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PhD Thesis

Diagnosis and treatment of acutely hospitalised patients with suspected community-acquired pneumonia – clinical and microbiological perspectives

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Thesis submitted for the degree of Doctor of Philosophy
at the University of Southern Denmark

By

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Motivation and acknowledgements

The work presented in this thesis was based at the Emergency Medicine Research Unit, Hospital Sønderjylland. In its realization, I owe much to remarkable individuals' invaluable support and inspiration. Their unique contributions have been pivotal to completing this dissertation.

Many are curious as to understand why a physiotherapist could be immersed in medicine and microbiology. Although my experience with numerous lung disease patients and the handling of sputum was not entirely foreign, it paled in comparison to the unprecedented scenarios that presented awaited me. Thus, I began immersing myself in a sea of knowledge. Driven by a yearning to fathom the true impact of my treatments on my patients' well-being. My quest for optimized health outcomes led me to broaden my horizons and I was given the opportunity to start my PhD in 2020. Few years before, I had the honor of meeting Helene Skjøt-Arkil and Christian Backer Mogensen. Their enthusiasm kept my vision ignited where simple sputum holds profound worth, guiding precise patient treatment and contributing to combating antibiotic resistance. My motivation for this work springs from a thirst for knowledge, a drive to enhance patient care, and collaborate with passionate, ambitious, visionary individuals. Furthermore, I believe that integrating perspectives through interdisciplinary collaboration is the key to effectively addressing our healthcare challenges.

I have been privileged to have my very own "dream team" of mentors, and each of their contributions has been immensely valuable. Foremost, I would like to extend my heartfelt gratitude to my principal supervisor, Helene Skjøt-Arkil. Her unwavering belief in my abilities, constant encouragement, expert guidance, and invaluable lessons in research have been instrumental in my

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My PhD journey has enriched me with skills and insights that I am passionate about and eager to employ in the future, alongside forging new connections.

Mariana Bichuette Cartuliares

Aabenraa 2023

List of papers

Paper I

Cartuliares MB, Mogensen CB, Rosenvinge FS, Skovsted TA, Lorentzen MH, Heltborg A, Hertz MA, Kaldan F, Specht JJ, Skjøt-Arkil H *Community-acquired pneumonia – Use of clinical characteristics of acutely admitted patients for the development of a diagnostic model: A cross-sectional multicentre study* (BMJ Open, submittet 22/08/2023)

Paper II

Cartuliares MB, Rosenvinge FS, Mogensen CB, Skovsted TA, Andersen SL, Pedersen AK, Skjøt-Arkil H. *Expiratory technique versus tracheal suction to obtain good-quality sputum from patients with suspected lower respiratory tract infection: a randomized controlled trial.* Diagnostics (Basel). 2022; 12(10).

Paper III

Cartuliares MB, Mogensen CB, Rosenvinge FS, Skovsted TA, Andersen SL, Østergaard C, Pedersen AK, Skjøt-Arkil H. *The effect of point-of-care multiplex polymerase chain reaction of respiratory specimens on antibiotic treatment of patients acutely admitted with suspected community-acquired pneumonia in Denmark: A multicentre randomised controlled trial* (PLoSMedicine, submitted 21/06/2023, under review)

Additional work and papers

Cartuliares MB, Skjøt-Arkil H, Rosenvinge FS, Mogensen CB, Skovsted TA, Pedersen AK. *Effectiveness of expiratory technique and induced sputum in obtaining good-quality sputum from patients acutely hospitalized with suspected lower respiratory tract infection: a statistical analysis plan for a randomized controlled trial.* Trials. 2021;22(1):675.

Cartuliares MB, Skjøt-Arkil H, Mogensen CB, Skovsted TA, Andersen SL, Pedersen AK, et al. *Gram stain and culture of sputum samples detect only few pathogens in community-acquired lower respiratory tract infections: secondary analysis of a randomized controlled trial.* Diagnostics (Basel). 2023;13(4).

Cartuliares MB, Søgaard SN, Rosenvinge FS, Mogensen CB, Hertz MA, Skjøt-Arkil H. *Antibiotic prescription pattern at the Emergency Department: a descriptive study from a country with low antimicrobial resistance.* (Antibiotics, Submitted 13/07/2023, under review)

Skjøt-Arkil H, Heltborg A, Lorentzen MH, Cartuliares MB, Hertz MA, Graumann O, et al. *Improved diagnostics of infectious diseases in emergency departments: a protocol of a multifaceted multicentre diagnostic study.* BMJ Open. 2021;11(9):e049606.

List of abbreviations

AMR	Antimicrobial resistance
AUC	Area under the curve
BAL	Bronchoalveolar lavage
CAP	Community-acquired pneumonia
CI	Confidence intervals
CRP	C-reactive protein
CT	Computer tomography
CXR	Chest X-ray
ED	Emergency department
FET	Forced expiratory technique
FLUS	Focused lung ultrasound
HAP	Hospital-acquired pneumonia
HCAP	Hospital care-associated pneumonia
HIV	Human immunodeficiency
HRCT	High-resolution computer tomography
IgA	Immunoglobulin A
IL-6	Interleukin-6
INDEED	Infectious Diseases in EmErgency Departments
IQR	Interquartile range
IRR	Incidence rate ratio
IS	Induced sputum
KL6	Krebs von den Lungen 6

LASSO	Least absolute shrinkage and selection operator
LRT	Lower respiratory tract
LRTI	Lower respiratory tract infection
LUS	Lung ultrasound
NAAT:	Nucleic acid amplification tests
NGAL	Neutrophil gelatinase-associated lipocalin
OR	Odds ratio
PCR	Polymerase chain reaction
PCT	Procalcitonin
PICO	Population, intervention, comparison, outcome
POC	Point-of-care
PPI	patient and public involvement
RCT	Randomised controlled trial
REDCap	Research Electronic Data Capture
SCO	standard care only
suPAR	Soluble Urokinase Plasminogen Activator Receptor
TS	Tracheal suction
ULDCT	Ultra-low doses of computer tomography
UN	United Nations
URT	Upper respiratory tract
URTI	Upper respiratory tract infection
VAP	Ventilator-acquired pneumonia
YKL-40	tyrosine (Y), lysine (K) and leucine (L) protein

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Source of the icons reused in the thesis signalling studies I and III: Free download Icons from https://thenounproject.com/ (modified: symptoms icon 330674 and medication icon 4238926).

TABLE OF CONTENT

SUMMARY IN ENGLISH

Community-acquired pneumonia (CAP) is a leading cause of mortality worldwide, associated with high morbidity and hospital costs. CAP is one of the most common infections diagnosed in Emergency Departments (ED), requiring timely antibiotic treatment within a few hours from patient admission. The CAP diagnosis is often based on uncertain history, questionable diagnostic methods and unspecific blood tests. This challenges clinicians to make correct early diagnoses, with misdiagnosis risking adverse events, poorer patient outcomes, increased healthcare costs and the overuse of broad-spectrum antibiotics. This, in turn, contributes to the increased development of resistant bacteria, thereby threatening future treatment possibilities.

This PhD thesis aimed to investigate potential improvements to the CAP diagnostic process within the first hours of acute admission. An improved diagnostic process would use rapid and precise diagnosis methods to support rational, targeted antibiotic prescriptions, preventing poor patient outcomes and antimicrobial resistance (AMR).

In study I, it was hypothesised that well-defined clinical characteristics could assist ED physicians in making an earlier, more accurate CAP diagnosis. The study design was a cross-sectional diagnostic, predictive study. The study identified the clinical characteristics of patients with CAP, developed a diagnostic model and compared the model's performance to the ED physician's initial assessment. The model yielded 13 predictors, all recognised and supported by published literature. The performance and calibration of the model were good but did not outperform the initial tentative

diagnosis made by the ED physicians. The addition of new diagnostic tools will be essential in future diagnostic models.

In study II, it was hypothesised that expiratory techniques (forced expiratory technique and sputum induction) were non-inferior to tracheal suction for collecting good-quality sputum samples from patients with suspected lower respiratory tract (LRT) infection in the ED. The number of adverse events between groups was compared, and patient experiences of sampling methods were investigated. For this thesis, additonal microbiological results of good-quality LRT specimens are described. The study was an open-label, parallel-armed, non-inferiority randomised controlled trial (RCT). Results showed that tracheal suction had approximately a twofold likelihood of ensuring a good-quality specimen compared with expiratory techniques. However, often good-quality samples had low microbiological yields. Although there were no differences when adverse events between the two sampling methods were pooled and compared, patients allocated to the expiratory technique reported a more positive experience than patients allocated to tracheal suction.

In study III, it was hypothesised that point-of-care polymerase chain reaction (POC-PCR) testing of LRT samples from suspected CAP patients would increase the proportion of patients treated with no or narrow-spectrum antibiotics compared with standard care only (SCO), which included routine culture and targeted-specific PCR if requested by the ED physician. The study compared the length of stay (LOS), intensive care unit (ICU) admission, mortality and readmissions between groups. Additional descriptive analysis was completed on bacteria and viruses from the microbiological analyses of the LRT specimens. The study was a multicentre, open-label, parallel-armed superiority RCT. Adding POC-PCR to the diagnostic setup did not increase

the number of patients treated with narrow-spectrum or without antibiotics, but the results indicated that patients in the POC-PCR group received earlier and more targeted antibiotic treatments. Compared with culture, POC-PCR identified more bacteria and viruses, including common CAP pathogens. No statistical differences between POC-PCR and SCO groups were observed for mortality, readmissions, ICU admissions or LOS.

In conclusion, this thesis reflects the challenges of diagnosing CAP and provides new insight into optimising the diagnostic process. These three studies contribute vital information and knowledge to future research and implementation strategies targeting the improvement of CAP diagnosis.

SUMMARY IN DANISH

Samfundserhvervet lungebetændelse er en af de førende årsager til død på verdensplan og er forbundet med høj morbiditet samt betydelige hospitalsomkostninger. Samfundserhvervet lungebetændelse forekommer hyppigt på fælles akutmodtagelsen (FAM), og en rettidig antibiotikabehandling inden for de første timer af patientens indlæggelse er afgørende for at undgå forværring af tilstanden eller i værste tilfælde død. Diagnosen stilles ofte ud fra usikker sygehistorik, tvivlsomme diagnostiske metoder og uspecifikke blodprøver. Dette udfordrer klinikere i at stille den korrekte diagnose tidligt. En fejldiagnosticering vil øge risikoen for længere behandlingsforløb, bivirkninger, dårligere patient outcomes, øgede sundhedsomkostninger og overforbrug af antibiotika. Det vil bidrage til øget udvikling af resistente bakterier og dermed true fremtidige behandlingsmuligheder ikke kun lokalt, men globalt.

Formålet med denne ph.d.-afhandling er at undersøge potentielle forbedringer i den diagnostiske process for samfundserhvervet lungebetændelse inden for de første timer efter akut indlæggelse med fokus på mikrobiologi og kliniske karakteristika. Afhandlingen bygger på tre studier, der karakteriserer patienter med lungebetændelse, undersøger hvordan en nedre luftvejsprøve af god kvalitet bedst tages, og om en hurtig-test af nedre luftvejsprøve har en effekt på hvilket antibiotika, der ordineres.

I studie I blev det antaget, at veldefinerede kliniske karakteristika af en population som den ser i dag, kan hjælpe FAM-læger med at stille en tidligere og mere præcis diagnose af lungebetændelse. Studiedesignet var et tværsnits- og diagnostisk prædiktionsstudie. Studiet identificerede kliniske karakteristika for patienter med lungebetændelse, udviklede en diagnostisk

model og sammenlignede modellens ydeevne med FAM-lægens tentative vurdering. Modellen resulterede i 13 prædiktorer alle allerede anerkendt og understøttet af publiceret litteratur. Ydeevnen og kalibreringen af modellen var god, men overgik ikke den tentative diagnose stillet af FAM-lægerne. Tilføjelsen af nye diagnostiske værktøjer vil være afgørende i fremtidige diagnostiske modeller.

I studie II blev det antaget, at eksspirationsteknikker (forceret eksspirationsteknik kombineret med saltvandsinhalationer) ikke var værre sammenlignet med trakealsugning for at opnå nedre luftvejsprøver af god kvalitet fra akut indlagte patienter med mistanke om infektion i de nedre luftveje. Bivirkninger og patientoplevelser i forbindelse med prøvetagningen blev sammenlignet mellem grupperne, og mikrobiologiske resultater af nedre luftvejsprøver, som var vurderet af god kvalitet undersøgt. Studiet var et ikke-blinded, parallelgruppe, non-inferiørt randomiseret kontrolleret studie. Resultaterne viste, at patienter randomiseret til trakealsugning havde næsten dobbelt så stor sandsynlighed for at levere en prøve af god kvalitet sammenlignet med patienter, som udførte ekspirationsteknikker. Vi fandt få mulige pathogener fra de mikrobiologiske prøver. Der var ingen forskel mellem grupperne i forhold til bivirkninger. Patienter allokeret til ekspirationsteknik-gruppen rapporterede en mere positiv oplevelse end patienter allokeret til trakealsugning.

I studie III blev det antaget, at point-of-care (POC) polymerase-kædereaktion (PCR) test af nedre luftvejsprøver fra patienter med mistanke om samfundserhvervet lungebetændelse vil øge andelen af patienter behandlet med smalspektret antibiotika eller ingen antibiotika sammenlignet

med standardbehandling (SCO) alene. SCO inkluderede rutinedyrkning og målrettet specifik PCR efter lægens anmodning. Studiet sammenlignede hospitalsindlæggelsestid, indlæggelse på intensiv, død og genindlæggelser inden for 30 dage mellem de to grupper. Yderligere blev bakterier og vira fra de mikrobiologiske analyser af nedre luftvejsprøver beskrevet. Studiet var et multicenter, åben-label, parallelgruppe, superiørt randomiseret kontrolleret studie. Tilføjelse af POC-PCR til det diagnostiske setup resulterede ikke i flere patienter behandlet med smalspektret eller ingen antibiotika, men resultaterne indikerede, at patienter i POC-PCR-gruppen modtog tidligere og mere målrettede antibiotikabehandlinger. Sammenholdt med dyrkning identificerede POC-PCR flere bakterier og vira, herunder almindelige lungebetændelsespathogener. Der blev ikke observeret statistiske forskelle mellem POC-PCR- og SCO-grupper i forhold til død, genindlæggelser inden for 30 dage, indlæggelse på intensiv eller hospitalsindlæggelsestid.

Denne ph.d.-afhandling afspejler de mangeartede udfordringer ved diagnosticering af samfundserhvervet lungebetændelse og giver ny indsigt i mulighederne for optimering af den diagnostiske proces. De tre studier bidrager med værdifuld information og viden til fremtidige forsknings- og implementeringsstrategier med fokus på forbedring af diagnostik af lungebetændelse.

1. INTRODUCTION

In 1918, pneumonia was one of the most widespread acute diseases and a major cause of death. Sir William Osler described pneumonia as the 'Captain of the men of death' (1). Then, in 1928, Alexander Fleming discovered penicillin and antibiotics were used to treat serious infections, saving millions of lives, extending life expectancy, playing an essential role in medical practice and changing the outcome of several bacterial infections (2-5). However, quite soon after his discovery of penicillin, Alexander Fleming provided this warning:

> *the public will demand a preparation (penicillin) ... then will begin an era ... of abuses … The microbes are educated to resist penicillin and … can be passed to other individuals … until they reach someone who gets a septicemia or a pneumonia which penicillin cannot save. In such a case, the thoughtless person playing with penicillin treatment is morally responsible for the death of the man who finally succumbs to infection with the penicillin-resistant organism. I hope the evil can be averted* (6).

As noted in several studies and systematic reviews, there is no doubt that the irrational and extensive use of antibiotics has led to an alarming situation where antibiotic consumption is associated with the development of antimicrobial resistance (AMR) (2, 4, 7-9) causing millions of death worldwide (10, 11). This situation brings antibiotic resistance to the top of the World Health Organization's list of the biggest threats to global health (12). This increasing concern has resulted in the development of several antibiotic stewardships focusing on the appropriate treatments to reduce overall antibiotic prescriptions and prevent the development of AMR (13-18).

This thesis focuses on community-acquired pneumonia (CAP) because it is the most common lower respiratory tract infection (LRTI) and one of the most frequent infections presented in Emergency Departments (ED) (19). CAP requires timely antibiotic treatment within a few hours from patient admission (20) to avoid serious complications such as bacteremia, sepsis, organ failure and death (21). However, the delivery of a rapid and targeted treatment is challenged. Difficulties in identifying the aetiology, the variability of clinical signs and symptoms, questionable diagnostic tools and unspecific blood tests all make the diagnosis of CAP difficult to determine (22-26). These factors contribute to clinical uncertainty or delayed diagnosis. Such uncertainties can lead to the overuse of broad-spectrum antibiotics (27, 28), resulting in poorer patient outcomes, extended hospital stays and increased healthcare costs (29).

The goal of this thesis is to contribute to the rapid and precise diagnosis of CAP, including the detection of causative pathogens of CAP. The detection of these causative pathogens has been identified as one of the most important needs in CAP research (30). This is because the provision of appropriate antibiotics targeting the etiological agent (14, 15) will contribute to better patient outcomes and prevent the emergence of AMR.

1.1 How the thesis was structured

This thesis comprises three studies that follow the patient through the diagnostic process during the first hours upon their admission at the ED. It focuses on the identification of clinical characteristics of patients with verified CAP (study I, paper I), investigates the quality of respiratory samples collected by different methods (study II, paper II), and measures the effects of rapid microbiological analysis of respiratory samples on antibiotic treatment (study III, paper III) (Figure 1).

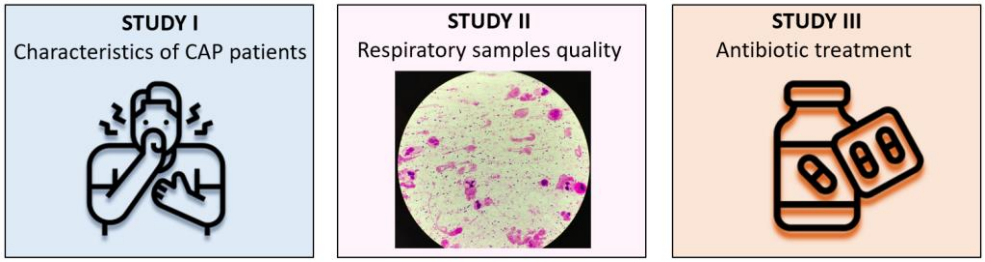


Figure 1: The investigation focus of the three papers in this thesis
Source: Free download Icons from https://thenounproject.com/ (modified: symptoms icon 330674, medication icon 4238926 and lung icon 1843622). Photograph of Gram stain by Steen L. Andersen.

The following section describes the diagnostic process model. It shows the importance of an accurate diagnosis and illustrates the complexity of improving diagnosis in healthcare. Chapter 2 describes the background of CAP epidemiology, pathophysiology, aetiology, definition, diagnosis and treatment. An outline of the rationale for the thesis, along with the study aim and objectives, follow in chapter 3, and the methods used for the three studies are described in chapter 4. A summary of the results and additional results of the studies are presented in chapter 5, followed by the discussion in chapter 6. The overall conclusion follows in chapter 7. Finally, the perspectives of this work are described in chapter 8.

1.2 Diagnostic process

Understanding the diagnostic process and what influences a precise and timely diagnosis and prevents diagnostic error is essential for improving diagnostics at the ED and providing the patient the opportunity for a positive outcome (31, 32). CAP is among the top 20 diseases associated with diagnostic error and one of the 15 diseases associated with serious misdiagnosis-related harms in EDs (33). Diagnostic errors vary but commonly include diagnoses that are unintentionally delayed, wrong, or missed; error in the administering of treatment; failure in the diagnostic process; or the failure to establish an accurate and timely diagnosis (32). Figure 2 presents the diagnostic process model inspired and adapted from 'Improving diagnosis in health care' (32). In summary form, CAP is acquired outside the hospital (34). Patients experience their symptoms and contact a primary care practitioner or the ED, where the diagnostic process starts. The diagnostic process includes gathering, integrating and interpreting information from patient history, physical exams and diagnostic testing, as well as through consultation. Failure in this process can contribute to diagnostic error. Success in the process will lead to an accurate and timely diagnosis of CAP, followed by a treatment plan and positive outcomes. Treatment results and patient and systems outcomes contribute to knowledge that can improve the diagnosis of CAP, and the multifaceted system influences the success or failure of the diagnostic process. The diagnostic process requires teamwork and collaboration from healthcare professionals, patients and their families. In the model, the three studies from this thesis have been added to visualise their focus. This model will be returned to in the discussion and perspectives of this thesis.

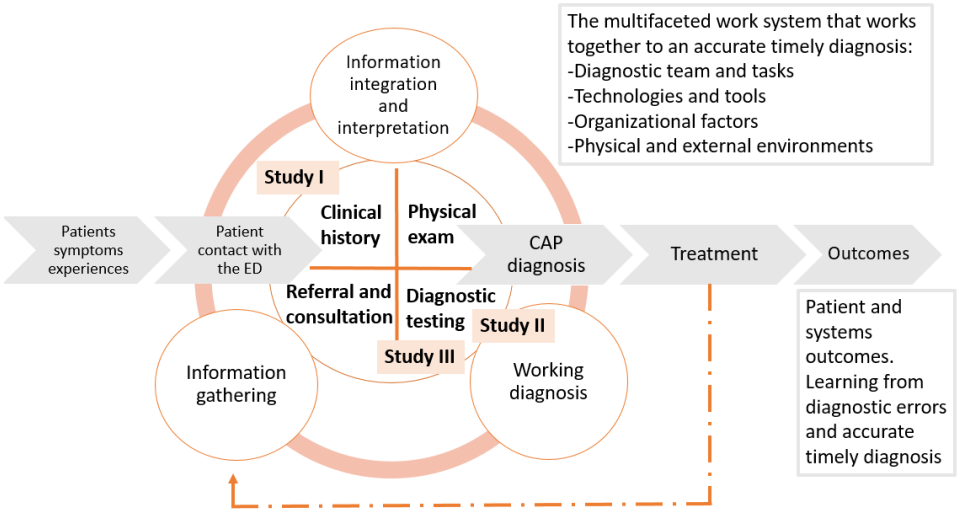


Figure 2: Overview of the diagnostic process cycle of information gathering, integration and interpretation. Source: Diagnostic process modified from 'Improving diagnosis in health care' (32).

2. COMMUNITY-ACQUIRED PNEUMONIA

2.1 Epidemiology and risk factors

LRTIs, including CAP, remain a major cause of morbidity and mortality and are associated with high economic costs and high rates of hospitalisation (35-38). LRTI is responsible for 2.6 million deaths worldwide and is the fourth-leading cause of death after ischemic heart disease, stroke and chronic obstructive pulmonary disease (COPD) (39). In Denmark, nearly 2,000 died from pneumonia in 2018 (40). The incidence of CAP is U-shaped. It primarily affects children younger than five and adults older than 65. Mortality risk increases with age (35, 41), with half of the adults who survive a CAP hospitalisation dying within five years (42) and one-third of patients with CAP presenting multimorbidities (43). The incidence of CAP is also higher in males compared with females (35-37), and the global prevalence of CAP among immunocompromised patients was reported to be 18% (44).

Systematic reviews have reported that socioeconomic factors; environmental exposures such as metals, dust and fumes; lifestyle factors; comorbid conditions and the use of medicaments are associated with an increased risk of CAP (45-47). These reviews describe that age (adjusted OR: 1.07, CI: 1.01 to 1.19 per year of increase) (47), gender (pooled OR: 1.30, CI: 1.27 to 1.33) (46), and educational level (< high school, crude OR: 2.70, CI: 2.03 to 3.60) (47) have all been associated with higher CAP risk. Contact with children (crude OR: 1.48, CI: 1.26 to 1.75) (45) increases the risk of CAP, but there is inconsistency in the literature regarding contact with pets (45, 46). There is robust evidence that current smokers have a higher risk of CAP compared with non-smokers (adjusted OR: 2.00, CI: 1.20 to 3.36)(45). Furthermore, higher alcohol consumption (> 41 g/day, crude OR: 1.59, (CI: 0.59 to 4.25),

malnutrition (OR: 2.14, CI: 1.58 to 2.70) (46), and poor dental status (adjusted OR: 2.78, CI: 1.60 to 4.40) (47) have all been associated with a significantly increased risk of CAP (45-47). The presence of comorbidities such as chronic respiratory disease, cardiovascular diseases, cerebrovascular disease, Parkinson's disease, dementia, dysphagia, chronic renal disease, HIV (human immunodeficiency) infection, liver disease or any previous hospitalisations all increased the risk of CAP up to fourfold, with the two top being chronic respiratory disease and chronic cardiovascular disease (45-47). Treatment with oral steroids (adjusted OR: 1.87, CI: 1.30 to 4.05) or immunosuppressive therapy (adjusted OR: 3.1, CI: 1.27 to 15.13) were reported as definitive risk factors for CAP (47).

2.2 Pathophysiology

CAP is an LRTI caused by colonised microorganisms that are mostly part of the upper respiratory microbiota in the nasopharynx and oropharynx. These microorganisms enter the LRT and alveoli, exploiting the lung's innate immune mechanism (48, 49). The development of CAP depends on the pathogen's virulence, the amount of inoculum and the immune system's ability to respond to host defences. Cytokines and local inflammatory markers are released, causing lung damage through an inflammatory process leading to an accumulation of white blood cells and fluid congestion. This leads to pus in the parenchyma. Transmission of pathogens from one individual to another can occur via direct or indirect contact, droplets and aerosols (48, 49).

Sputum works to protect the airway's epithelium against foreign pathogens, toxins and environmental particles. Wettability and adhesiveness are important physical surface properties contributing to the optimal interface between mucus and the epithelium. Together with rheological properties such

as viscosity and elasticity, wettability and adhesiveness facilitate the transportation of sputum in the airways by the ciliary or cough mechanism (50). A combination of unbalanced properties and weak host defences can lead to bacterial adherence in the airways, facilitating infection (50).

2.3 Microbial aetiology

The aetiology of CAP can be determined using several microbiological methodologies, including the assessment of sputum, blood, urine and pleural fluid samples (51). These methodologies are described in section 2.5, 'Diagnosing community-acquired pneumonia'.

The etiological agent for CAP is determined in less than 50% of the patients. When established, *Streptococcus pneumoniae* remains the most common cause of CAP worldwide, being identified in 33%–50% of all cases, followed by *Haemophilus influenzae* (7%–16%) and *Mycoplasma pneumoniae* 4%–11%. *Staphylococcus aureus* and *Enterobacterales* were equally identified in approximately 4%–10% of CAP. Thereafter, the rates for *Legionella pneumophila* and *Chlamydophila pneumoniae* range from 2%–8%. The incidence of less common pathogens is 0.8%–4.5% for *Pseudomonas aeruginosa* and 1.2%–3.5% for *Moraxella catarrhalis* (52).

Recently, because of technological advances with the use of polymerase chain reaction (PCR) analysis, studies have reported an increased established aetiology of CAP caused by viruses ranging from 30%-40% and viral/bacterial coinfection in 25%–35% of these cases (52).

2.4 Definition and clinical presentation

The definition of CAP is heterogeneous across guidelines and studies from different countries (17, 20, 34, 53-55). Pneumonia can be classified as CAP when acquired outside a hospital or healthcare institution in the previous fortnight (34). Hospital-acquired pneumonia (HAP) refers to pneumonia acquired at least 48 hours after hospital admission and includes ventilator-associated pneumonia (VAP) (occurring between 48–72 hours after intubation) (56). Aspiration pneumonia is considered part of CAP or HAP and occurs because of impaired swallowing, representing 5%–15% of all causes of CAP (57). Although hospital care-associated pneumonia (HCAP) is also described in the literature, it is not differentiated from CAP concerning aetiology (58).

The literature has generally characterised CAP by a combination of clinical symptoms consistent with an acute respiratory infection, such as cough, increased sputum production, thoracic pain, dyspnea and fever of more than 38°C. This definition is supported by a newly recognised lung infiltrate on chest imaging (17, 20, 34, 59-61).

The clinical presentation can differ, but abnormal vital signs such as lower oxygen saturation than 95%, a heart rate higher than 100 beats/min and a respiratory rate over 20/min are often observed in patients with CAP (62-64). Furthermore, abnormities from chest auscultation with a stethoscope, such as crackles and rhonchi, are widely described in the literature as clinical findings during patient assessment (61-65).

An aging population (66) and the presence of multimorbidities (43) contribute to a substantially different clinical picture of CAP in the elderly, further challenging ED physicians in the diagnosis of CAP. Atypical signs and

symptoms such as headache, gastrointestinal symptoms, malnutrition, fatigue, lethargy, falls, delirium and polypharmacy frequently appear (22, 59, 67-75). Moreover, the complexity of the clinical picture increases as older patients may present afebrile and lacking respiratory symptoms (54, 76).

2.5 Diagnosing community-acquired pneumonia

Challenges in diagnosing CAP in the ED do not only arise from the overlap of signs and symptoms among a large group of diseases. Clinical complexity and poor patient cooperation make physician evaluation more difficult. The ED physician's initial CAP diagnosis can differ from the discharge diagnosis by 18%–25% (26). Diagnostic uncertainty caused by the variability of symptoms could be mitigated if the physician could trust a precise diagnostic tool to support the initial diagnosis. However, the diagnostic tools available upon admission are nonspecific (77). They are described in the following sections.

2.5.1 Serum biomarkers

Several serological biomarkers with differential cut-offs have been studied to enhance the accuracy of diagnosing CAP. Nonetheless, most of the biomarkers tested in the acute setting are indicators of systemic inflammation and infection (78, 79). C-reactive protein (CRP) has been preferred for diagnosis of outpatient CAP because of its low cost, accuracy and availability. However, the accuracy of CRP differs, and its utility as a diagnostic marker for CAP or to guide antibiotic treatment is not consistently supported by the present evidence (63, 64, 80-82). Investigations of CRP alone or combined with other biomarkers, such as procalcitonin (PCT), indicate that it has been inefficient in helping physicians diagnose CAP (83). PCT has shown promising results as a diagnostic marker at the ED, showing a higher

diagnostic accuracy than CRP and leucocytes as well as a greater ability to differentiate CAP from other diagnoses and predict bacterial CAP (65). However, other studies found a lower diagnostic accuracy of PCT when compared with interleukin-6 (IL-6) and CRP (84), and also low reliability to guide antibiotic administration of CAP (85). Various biomarkers targeting local inflammatory reactions in the lungs as well as bacterial cell membranes have been investigated, but further understanding of the mechanisms and external validation are needed (86, 87). Currently, there are no biomarker-based algorithms specific for diagnosing CAP.

2.5.2 Imaging

Guidelines for diagnosing and managing CAP recommend the addition of chest X-ray (CXR) to support physicians in diagnosing CAP. CXR has become the standard imaging for patients suspected of CAP at the ED (17, 60). However, studies have shown low accuracy of the CXR compared with computer tomography (CT) and have suggested that patients with suspected CAP would benefit from CT to guide clinical decisions at the ED (24, 88, 89). Because it can provide a detailed image of the lung parenchymal, CT is the gold standard in diagnosing CAP and other specific diagnoses and abnormalities (90). However, CT has limitations, including cost, radiation exposure and the impossibility of performing CT at the bedside (88, 90, 91). Novel imaging diagnostic tools, such as ultra-low-dose CT (ULDCT) and lung ultrasound (LUS), have been investigated recently because of their lower or no radiation levels compared with CXR and CT (92). However, the results of a multicentre randomised clinical trial examining patient outcomes and healthcare efficiency enforce the current guidelines of not recommending the replacement of ULDCT for CXR in EDs (93). Even though LUS is radiation-free, rapid, easily available and can be performed at the bedside, results are

sonographer-dependent and the studies' methodologies are heterogeneous, resulting in unsure conclusions (77, 94, 95). Consequently, the CXR remains the routine imaging diagnosing CAP supported by guidelines (17, 60).

2.5.3 Microbiology sampling and analysis methods

Microbiology analyses of urine, blood, pleural fluids and sputum have been performed to identify the aetiology of CAP. Guidelines differ in recommending these analyses in the diagnostic of CAP in the ED (13-15, 17, 60). This section describes primary LRT specimens and briefly overviews the underlying evidence for microbiology analysis of urine, blood and pleural fluids.

2.5.3.1 Test of urine, blood and pleural fluid

Urinary antigen tests are rapid and can detect serogroup 1 *L.pneumophila* antigen (96) and all serotypes of *S.pneumoniae* cell wall (C-) polysaccharide by immunochromatographic technique (97, 98). Meta-analyses reported these tests useful for diagnosing CAP because of their higher sensitivity than culture (96, 99). However, these tests showed several limitations, and studies presented poor quality, mainly depending on patient selection (96, 97, 99, 100). Furthermore, in recent studies, urine antigen test for *S.pneumoniae* was found to be less sensitive and has not been shown to improve patient care or antimicrobial stewardship or lead to cost savings (97). Positive blood culture rates from EDs were reported low, ranging from 3% to 10% for all-cause of CAP, and therefore the clinical usefulness of these samples is limited and rarely results in an appropriate change in empiric therapy (101). Pleural fluid cultures obtained from pleura exudate (2.9%) are the diagnostic less frequently used in Europe for the diagnosis of CAP compared with blood (68.8%), sputum (63.8%) and urine (43.3%) (102). These points together

mean that urine, blood and pleural fluid specimen sources have limited contribution to the clinical management of CAP in ED.

2.5.3.2 Lower respiratory tract specimens

LRT specimens have been used for several purposes. These include i) the therapeutic purpose of facilitating airway clearance, especially for patients with chronic respiratory diseases (103); ii) the diagnostic purpose of supporting the presumptive diagnosis at admission (15, 104) and monitoring the development of infection to target the antibiotic treatment (13, 56); and iii) the surveillance of antimicrobial susceptibility and development of antibiotic-resistant organisms (13). In this thesis, the role of the LRT specimen collection is to contribute to diagnosing CAP and identifying targeted treatments.

2.5.3.2.1 Collection of lower respiratory tract specimens

LRT specimens can be obtained by several methods (105, 106). The most invasive procedures are those most likely to be free of contamination from the upper airways. These include transtracheal suction, transthoracic needle aspiration, bronchoalveolar lavage (BAL) and tracheal suction (TS) (51, 105, 107). Less invasive methods include induced sputum (IS) (108, 109) and self-expectoration (103, 106, 110, 111). Figure 3 shows an example of expectorated and tracheal specimens.

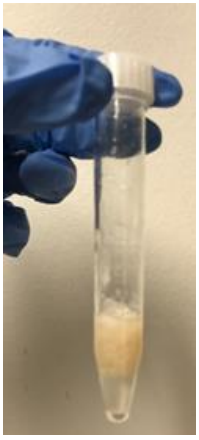


Figure 3: Expectorated sputum (left) and tracheal secretion (right)
Source: Photograph by Mariana B. Cartuliares.

The Region of Southern Denmark guidelines recommend TS and expectorated sputum if the specimen originates from the LRT (15). In the acute setting, the most frequent sampling method used to identify the aetiology of CAP is expectorated sputum (112). A big challenge is that a substantial percentage of patients are not able to expectorate spontaneously (112, 113). Another concern is that expectorated sputum might contain oropharyngeal contamination that may overgrow the actual pathogen, decreasing the microbiology analysis's diagnostic yield and generating misleading results (114). Therefore, it is recommended that the microbiological examination should be performed based on good-quality sputum samples from the LRT (115).

2.5.3.2.2 Quality of lower respiratory tract specimens

Because the quality of LRT specimens plays an essential role in identifying LRTI pathogens and targeting antibiotic treatment, recent international clinical guidelines highlight the importance of using good-quality sputum samples for microbiological diagnostics (13, 17, 60, 115). Good-quality sputum was defined in 1975 by Murray and Washington as samples with < 10 squamous epithelial cells (SEC) and > 25 polymorphonuclear leukocytes (PMNL) per low power field of view (10 × objective) observed by microscopic examination in a Gram-stained smear. SEC indicates the degree of oropharyngeal contamination and PMNL indicates inflammation. It was recommended that samples be screened for acceptability and that specimens with 10 SEC or more should be rejected for bacterial culture (116). Since then, studies have used differential criteria for screening sputum samples for acceptability before culture (117-120).

The identification of the etiological agent increased by over 40% when the microbiological analysis was based only on good-quality samples (52, 121). Furthermore, Gram stain from good-quality samples has reported higher diagnostic accuracy in diagnosing CAP (104, 122). These recent studies and meta-analyses highlighted the importance of obtaining good-quality specimens as a prerequisite for determining CAP aetiology and targeting treatment.

2.5.3.2.3 Gram stain and culture

Guidelines for the management of CAP recommend Gram stain and culture of sputum samples to monitor microbiological susceptibility and to assure the prescription of appropriate antibiotics to target the identified etiological agent (14, 15).

Assessed by microscopy, the Gram stain is a taxonomic analysis tool used for decades to identify and differentiate bacteria by their cell wall structure (123). Part of the sputum is placed on a microscope slide, and a second microscope slide is used to distribute the material on the surface. The smear is then heat-fixed and Gram stained. Gram-positive bacteria such as *S.pneumoniae* and *S.aureus* retain the initial purple stain (see example in Figure 4) because their cell wall contains little lipid, decreasing their permeability to organic solvents.

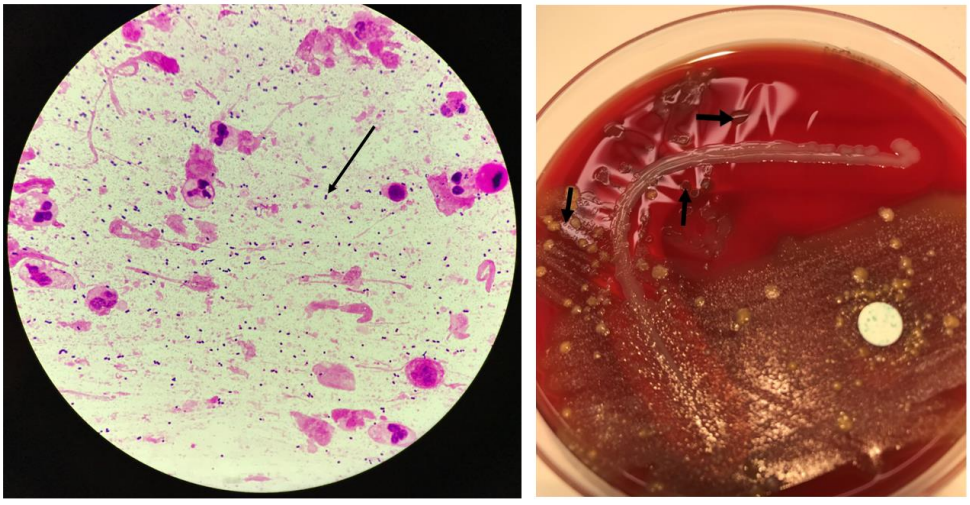


Figure 4: Example of Gram-stained good quality sputum (x 100 objective): Gram-positive diplococci (left) and culture of *Streptococcus pneumoniae* (right) Source: Photograph by Steen Lomborg Andersen.

In contrast, Gram-negative bacteria such as *H.influenzae* and *Enterobacterales* are decolourise and stained red (see example in Figure 5) because of the high lipid concentration of the cell wall permitting high permeability (123). Although the accuracy of the Gram stain has been extensively discussed, the method retains substantial support from the literature as adding value to the management of CAP patients with rapid results and targeted treatment (104, 122).

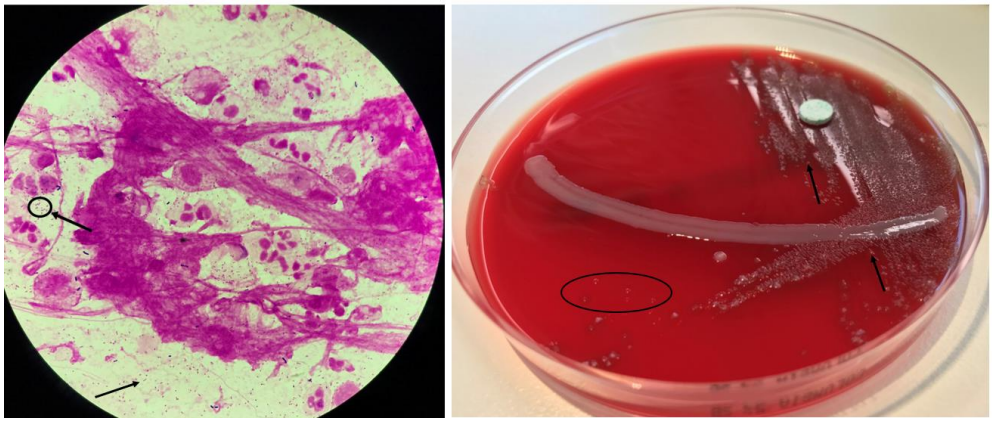


Figure 5: Example of Gram-stained good quality sputum (x 100 objective): small Gram-negative rods (left) and culture of *Haemophilus Influenzae* (right), including observed isolated symbiotic colonies (circle)
Source: Photograph by Steen Lomborg Andersen.

LRT specimens are cultured on media that contain components for optimal growth and cultivation of microorganisms. These components include a specific pH; a nutrient source; different compositions of nitrogen, carbon, minerals and other substances; and a solidifying agent for solid media (124). There are different types of media developed for various purposes. Sheep blood agar medium is used for general purposes and can detect most

organisms. MacConkey agar is used as a differential medium for the isolation of enteric pathogens and as selective for Gram-negative bacteria inhibiting other organisms, enriched media allow fastidious microorganisms to grow, and other specialised media are developed to isolate *Legionella* species. The LRT inoculum is streaked over the agar surface and inoculated (124). In our study settings, blood agar plates are inoculated with a *Staphylococcus* streak to allow growth of *H.influenzae* and are incubated in a 5% CO_2 atmosphere, other plates at 35 ºC in normal atmospheric conditions. Results are generally available after 48 hours of incubation. Thereafter, Matrix-Assisted Laser Desorption/Ionization-time of flight (125) is used to recognise the 'bacteria fingerprint' and identify specific pathogens. This turnaround time of two days for culture results is a limitation that hinders an early diagnosis and appropriate treatment when patients are acutely hospitalised with CAP. At this time, patients are moved to other wards or discharged home (126).

Other challenges related to Gram stain and culture are that the sensitivity of these analyses decreases if patients are treated with antibiotics. Such treatment results in false-negative findings and overgrown Gram-negative pathogens (118, 127-129). Furthermore, common viral etiologies of CAP are not possible to distinguish from bacterial infections without the aid of additional diagnostic testing (130, 131).

2.5.3.2.4 Polymerase chain reaction

PCR tests are molecular tests that amplify a pathogen's nucleic acid allowing rapid and accurate identification. The first molecular methods were used to identify *M.pneumoniae*, *L.pneumophila*, *C. pneumoniae* and viruses because these agents were negative assessed by Gram stain and were difficult to grow on conventional media (100). The development of single nucleic acid

amplification tests (NAAT) to multiple multiplex NAATs (132) has increased with other PCR methods. Recently, syndromic molecular panel essays detecting several targets and antimicrobial resistance genes in a single reaction have been used to assist in diagnosing LRTI. These molecular panels are highly sensitive and capable to detect several targets, including bacteria and viruses, with results available within a short time frame (131, 133). Because viruses account for 20%–40% of CAP cases (55, 134, 135), these PCR panels can contribute to reducing antibiotic prescriptions as patients with a viral CAP may be managed differently (135, 136). A study reported that molecular testing of LRT specimens improved the detection of pathogens from CAP patients by 48% when compared with culture methods. These results are consistent even with antibiotic administration before specimen collection (132). However, PCR may detect commensals from the upper airway microbiota as it does not distinguish between colonisers and pathogens. This highlights the importance of obtaining good-quality samples from the LRT and the need for a professional interpretation of the results. Figure 6 shows the preparation for PCR analysis of sputum.

Figure 6: Preparation for PCR analysis of sputum in the Biofire® FilmArray® Pneumonia Panel plus (Biomérieux, Marcy l'Etoile, France)
Source: Photograph by Mariana B. Cartuliares.

2.6 Severity assessment

Triage is a priority clinical assessment tool in a crowded ED and is used upon patient admission to assess the severity and urgency of a patient‘s condition to optimally allocate resources (137, 138). The Danish Emergency Process Triage (DEPT) is mostly implemented in Danish EDs (139). DEPT was adapted and modified from the Adaptive Process Triage (ADAPT) developed in Sweden (140) and shares core similarities with widespread standardised 5-level triage systems (137). Patients are categorised into five triage levels based on vital signs and a presenting complaint algorithm (141).

Several tools are specific for the severity assessment in the management of CAP to support ED physicians in their clinical and site of care decisions. These tools have shown similar prediction performance on mortality from CAP patients (142). The CURB-65 is recommended by the European guidelines (17, 60), including in Denmark (15). Encompassing only five items, with a single point awarded for each, the CURB-65 score easier to remember and manage than, for example, the pneumonia severity index, which contains several items and is more complex to use, especially in a busy ED (142). The CURB-65 definition, risk stratification, prediction of mortality for each group and recommended site of care are presented in Table 1 (143). The CURB-65 severity assessment of CAP is included in the antibiotic treatment guidelines to guide the administration of the choice of antibiotic therapy (15).

Table 1: CURB-65 definition, risk stratification, prediction of mortality for each group and recommended site of care

CURB-65			**Points to be added to the score**
Confusion			1
Urea (> 7 mmol/L)			1
Respiratory rate ≥ 30 bpm			1
Blood pressure (≤ 90 mmHg systolic or ≤ 60 mmHg diastolic)			1
Age of > **65** years			1
CURB-65 score	**Risk group**	**30-day mortality**	**Management**
0–1	Low mortality risk	< 2%	Treatment outside the hospital
2	Intermediate mortality risk	9%	Supervised treatment at the hospital
≥ 3	High mortality risk	> 20%	Urgent hospital admission

2.7 Treatment

2.7.1 Antimicrobial Treatment

Antibiotics are the mainstay treatment for CAP and should be started within a few hours as soon as the presumptive diagnosis has been made. The therapy is empirical until diagnostic test results become available to target the treatment accordingly. The clinical guideline pathway for the management of antibiotics is presented in Table 2.

Table 2: Clinical guideline timeline pathways

30 minutes	Within 4 hours	Within 48 hours	Within day 5
-Clinical assessment -LRT specimens obtained	-Indication for antibiotics? -Narrow empiric treatment?	-Adjustment of antibiotic administration	-Follow-up and adjustment of antibiotic administration -Re-assessment every 3rd day

There are different recommendations for the choice of empiric treatment in patients with CAP depending on disease severity, comorbidities, allergies, individual risk factors and antimicrobial susceptibility (144). Because increasing antibiotic consumption is strongly associated with increasing AMR (7), the initial treatment should be individualised in accordance with the most likely etiological agent from local epidemiological data.

Europe has a widespread geographic difference in AMR, with resistance in *S.pneumoniae* and *H.influenzae* being lower in northern countries than in southern Europe (7, 145, 146). In Denmark, *S.pneumoniae* and the majority of *H.influenzae* (25% ampicillin resistant) are susceptible to penicillin and ampicillin (147). This means that penicillin/ampicillin remains the first choice of empirical treatment of CAP (15).

Empiric treatment for the management of CAP in accordance with clinical guidelines (15) is presented in Table 3. Targeted antibiotic treatment is given specifically against a detected bacterial pathogen identified by culture. Guidelines for targeted treatment are shown in Appendix A.

Table 3: Empiric treatment guidelines of CAP of the Region of Southern Denmark

Severity of CAP	First choice	Penicillin allergy	Therapy duration (iv* and oral)
CURB-65: 0–2	Benzylpenicillin 1.2g (2 mill.IE) × 4 iv. Or Phenoxymethylpenicillin 0.6g (1 mill.IE) × 4 oral	Cefuroxime 1.5g × 3 iv. Or Roxithromycin 300mg × 1 oral	5 days
CURB-65 ≥ 3	Benzylpenicillin 1.2g (2 mill.IE) × 4 iv. + Azithromycin† 500mg × 1 iv.	Cefuroxime 1.5g × 3 iv. + Azithromycin 500mg × 1 iv.	7 days
CURB-65 ≥ 3+⊕	Piperacillin-tazobactam 4/0.5g × 3 iv. + Azithromycin 500mg ×1 iv.	Cefuroxime 1.5g × 3 iv. + Azithromycin 500mg × 1 iv.	7 days

*Intravenous route. † Azithromycin: The treatment is extended only if PCR is positive for *Legionella pneumophila*, *Mycoplasma pneumoniae* or *Chlamydophila pneumoniae*. Azithromycin (500 mg iv.) has approximately 2–4 days therapeutic coverage. ⊕CURB-65 ≥ 3+: radiological involvement of multiple lung lobes, or hypoxia with O_2 saturation < 92%, or sepsis.

2.7.2 Antiviral treatment

Uncomplicated virus infection usually improves with or without antiviral treatment, and these treatments have limited effect in prophylactic treatment and asymptomatic influenza (148). The antiviral neuraminidase inhibitor is recommended to treat seasonal and pandemic influenza as the first-line treatment of patients with confirmed or suspected influenza that are hospitalised, patients with severe, complicated or progressive disease and patients with a high risk of complications from influenza infection. The benefit of neuraminidase inhibitor is greatest within the first 48 hours after symptoms onset, shortening the time from symptoms to clinical improvement

(149). Vaccination remains the basis for preventing and controlling influenza (150).

2.7.3 Adjunctive treatment

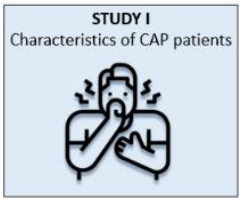
In addition to antibiotics, studies have investigated the benefits of adjunctive corticosteroid therapy in patients with severe bacterial CAP. This is because systemic corticosteroids may improve the inflammatory response in cases of inflammation and organ dysfunction. Although the results from a recent systematic review have reported that the use of corticosteroids was associated with a reduction in the need for mechanical ventilation from aggravation of CAP, results indicate higher rates of hospital readmission (151). No association was found between corticosteroid use and mortality, treatment failure or adverse events (151). Other studies found a lower risk of death for patients receiving hydrocortisone during intensive care unit (ICU) admission (152). However, there is no consensus from guidelines and studies regarding the optimal type, dose and duration of corticosteroids; these factors have not been determined (151).

3. AIM, HYPOTHESIS, AND OBJECTIVES

The previous section has described the background, definitions and management of patients hospitalised with CAP at the ED. It has shown that despite improvements in medical care, awareness of AMR and the development of guidelines, CAP still exhibits high mortality and morbidity worldwide, and that there are challenges in determining the diagnosis. This section outlines the aim of the thesis and provides a rationale for each study along with its hypothesis and objectives.

The aim

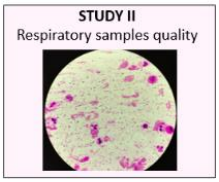
This PhD thesis has investigated potential improvements in the CAP diagnostic process within the first hours of acute admission. The aim is to facilitate a *rapid* and *precise* diagnosis that supports rational antibiotic prescriptions with targeted treatment and prevents poor patient outcomes and the development of AMR. The investigation has included three studies from a clinical and microbiological perspective.



Study I focused on the first clinical assessment of the patient upon arrival at the ED. As has been described, CAP is difficult to distinguish from other infections and respiratory conditions, and the population is changing, getting older and becoming more multimorbid. An investigation of an improved diagnostic model tailored to the current population is needed for accurate and timely CAP diagnosis.

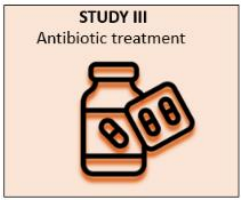
Study I hypothesised that well-defined clinical characteristics could assist an ED physician in making an earlier and more precise CAP diagnosis. The objectives were to: i) identify clinical characteristics of patients with CAP, depending on whether the physician suspected an infection or a CAP

diagnosis; and ii) develop and evaluate a diagnostic model capable of identifying patients with CAP among patients suspected of infection and compare the model performance to the initial assessment of the physician.



If the ED physician suspects the patient to have an LRTI at the anamnesis, a respiratory sample is obtained to confirm the presumptive suspicion of LRTI. Identification of the etiological agent supports the diagnosis to target antibiotic therapy. Many patients, however, are not able to deliver a specimen, and when successfully obtained, specimens are often of poor quality. The most effective method to collect a representative sample from the LRT remains uncertain.

Study II hypothesised that expiratory technique – forced expiratory technique (FET) and IS – was non-inferior to TS in collecting good-quality sputum samples from patients with suspected LRTI in an acute medical ward. The objectives were to: i) compare the effect of forced expiratory technique and induced sputum (FETIS) with TS in collecting good-quality respiratory samples from patients with suspected LRTI admitted at the ED, ii) compare the number of adverse events between the two groups, and iii) investigate the difference in patient experiences from the sampling methods.



After obtaining a respiratory sample, the revision of the empirical therapy is challenged by the low detection of pathogens by Gram stain and culture and the long turnaround for the results. This can increase the risk of misdiagnosis and inappropriate antibiotic administration.

Study III hypothesised that point-of-care polymerase chain reaction (POC-PCR) testing of sputum samples from suspected CAP patients would increase the proportion of patients treated with no or narrow-spectrum antibiotics. The objectives were to: i) compare the effect of POC-PCR testing of sputum from suspected CAP patients on the prescriptions of antibiotic treatment with standard care only (SCO) and ii) investigate if the addition of POC-PCR testing to the diagnostic setup affects the length of stay (LOS), ICU admission, mortality or readmissions.

4. METHODS

To ensure adequate information and transparency in the reporting of the studies, the 'Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis' (TRIPOD) statement (153) was applied for study I, and the Consolidation Standard of Reporting Trials (CONSORT) guidelines for parallel-group randomised trials (154) applied for studies II and III. The reported guideline checklists are presented following the supplementary material for the respective papers.

The published study protocols and statistical analysis plans for the three studies (155, 156), as well as papers I to III include detailed descriptions of the methods, including sampling methods for LRT specimens, microbiological analyses and statistics. All three studies were registered in ClinicalTrials.gov.

A structured literature search strategy for the three studies was based on block searching and applied the PICO (population, intervention, comparison, outcome) typology with Boolean operators (157). Each block in the search strings included controlled subject headings unique to each database, e.g. MeSH words combined with free text words with relevant truncation. The search accepted Danish, English, Spanish, French and Portuguese articles. The search was conducted at the start of the study and repeated in connection to article writing and during the writing of this thesis. A more explorative literature search, including grey literature and backwards and forwards citation searching throughout the entire work process. Additionally, an expert in literature search was consulted to ensure a precise strategy. The literature search strategy for all studies is presented in the Appendix B.

4.1 Study design and setting

An overview of the study design, setting, period, population and outcome of the three studies is presented in Figure 7. The data and participants for studies I and III originated from the multifaceted INDEED-study (Infectious Diseases in EmErgency Departments) (155). Study II was an independent study investigating the sampling method to be used in study III.

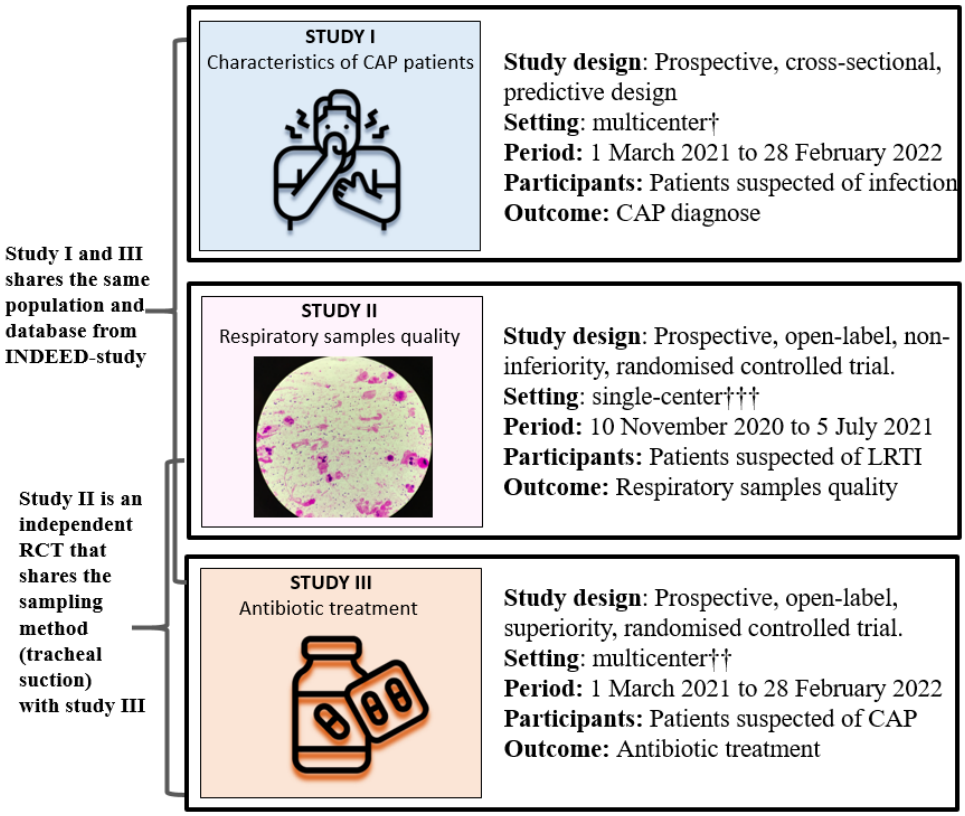


Figure 7: Studies design, setting, period, participants and outcome from the three studies included in this thesis
†included four EDs from three hospitals: Hospital Sønderjylland (Aabenraa and Sønderborg), Hospital Lillebælt (Kolding), and Odense University Hospital (Odense).
††Included two EDs (Aabenraa and Sønderborg) from the Hospital of Sønderjylland.
†††Included three EDs from three hospitals: Hospital Sønderjylland (Aabenraa), Hospital Lillebælt (Kolding), and Odense University Hospital (Odense).
Source: Free download Icons from https://thenounproject.com/ (modified: symptoms icon 330674 and medication icon 4238926). Photograph of Gram stain by Steen L. Andersen.

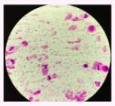
4.2 Recruitment

All three studies had identical setups concerning recruitment. Six experienced project assistants were responsible for the recruitment for studies I and III (three physicians, two last-year medical students and a physiotherapist) and study II (five nurses and a physiotherapist). A project assistant recruited patients on weekdays by consecutively identifying eligible patients through the local logistic system at the ED and obtaining verbal and written informed consent.

4.3 Participants

Inclusion and exclusion criteria for each study are presented in Table 4.

Table 4: Population for each study

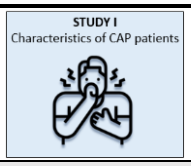
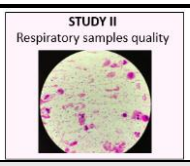
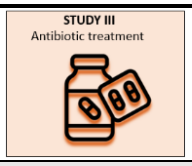
Studies	STUDY I Characteristics of CAP patients 	STUDY II Respiratory samples quality 	STUDY III Antibiotic treatment 
Inclusion criteria			
Adult patients (≥ 18 years)	**X**	**X**	**X**
Admission to the ED	**X**	**X**	**X**
Suspected of infection by the ED physician	**X**		
Suspected LRTI by the ED physician		**X**	
Suspected CAP by the ED physician			**X**
Exclusion criteria			
Urgent lifesaving treatment needed	**X**	**X**	**X**
Transferal to ICU	**X**	**X**	**X**
Sputum samples not obtained			**X**
Admission within the last 14 days	**X**	**X**	**X**
Verified COVID-19 infection at admission	**X**		
Severe immunodeficiency†	**X**	**X**	**X**

† Immunodeficiencies: HIV positive, with a cluster of differentiation 4 cell count < 200 or patients treated with immunosuppressive medicine (Anatomical Therapeutic Chemical classification L04A), corticosteroid treatment (> 20 mg/day prednisone or equivalent for > 14 days within the last 30 days) or chemotherapy within 30 days.

4.4 Data collection and data source

The project assistants were responsible for all data collected in the three studies. Data collection and data sources are presented in Table 5.

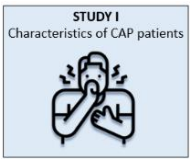
Table 5: Data collection and data sources for each study

Studies			
Data collection			
Collection of respiratory samples		**X**	**X**
Performing POC-PCR analysis			**X**
Registered in the REDCap* database (a)	**X**		**X**
Registered in the REDCap* database (b)		**X**	
Data sources			
Patient interview: Demographic data, patient symptoms, lifestyle factors	**X**		**X**
Patient medical record: clinical parameters, comorbidities	**X**		**X**
REDCap* database: outcome	**X**		
Patient medical record: outcome		**X**	**X**
Patient medical record: Demographic data, clinical parameters, comorbidities		**X**	
At bedside: symptoms aggravation, patient sampling method experience		**X**	

*REDCap (Research Electronic Data Capture) (158, 159). (a) Study I and III shared the same database, and (b) study II had an independent database.

4.5 Procedure, outcome and statistical analysis

Because procedures, outcomes and statistical analysis were different between the studies, they are presented separately in this section.



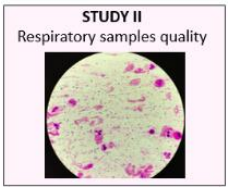
Study I: Community-acquired pneumonia – use of clinical characteristics of acutely admitted patients in a diagnostic model: a cross-sectional multicentre study

Procedure: Potential predictors were selected from the literature (45-47, 62, 80) after discussion among specialists and the project group and before the project commencement. A detailed description of the pre-specified potential predictors (n = 70) with their measurement unit, groups, cut-offs and consideration of inclusion are presented in the supplementary material for paper I. Characteristics of patients with CAP were collected upon arrival at the ED, and the project assistants collecting data were blinded to the final diagnosis.

Primary outcome: The diagnosis of CAP was assessed by an expert panel. The expert panel consisted of a specialist in emergency and infectious medicine at each site. The specialists determined the CAP diagnosis based on all information available in the patient's medical record and from the study database within the first week after admission, including CXR and chest CT. Disagreements were discussed until a consensus was reached.

Sample size: The study sample size was estimated based on data from Hospital Sønderjylland. It was estimated that at least 700 patients from the three hospitals admitted with suspected infection had to be included in the study.

Statistics: To examine the unadjusted association between each candidate predictor and the outcome CAP, extensive univariate logistic regression analyses of the 70 potential predictors were performed for descriptive purposes. An exploratory approach using the least absolute shrinkage and selection operator (LASSO) was performed, and a random split sample was used for the internal cross-validation. In addition, the receiver-operator characteristic (ROC) curve, the area under the ROC curve (AUC), and calibration were conducted to estimate the accuracy and performance of the model. The best threshold criterion was chosen for the predicted probability of the ROC curve, the sensitivity, specificity and positive and negative predicted values were calculated to illustrate the model effects, and a CAP-score was developed. Age ≥ 75 was considered an effect modifier based on studies showing differences in symptoms and signs for a CAP diagnosis in the elderly (67-69, 75). The ED physician's diagnostic accuracy was assessed by the sensitivity, specificity and positive and negative predicted values.



Study II: Expiratory technique versus tracheal suction to obtain good-quality sputum from patients with suspected lower respiratory tract infection: a randomized controlled trial

Procedure: Eligible patients were randomly assigned to i) TS procedure (usual care) or ii) FET and IS (intervention). An independent data manager generated the sequence using random block sizes of six without stratification. Randomisation was performed by project assistants using REDCap's computer-generated randomisation tool (158, 159). Randomisation was performed before sputum sample collection. Allocation concealment was ensured as the project assistants did not have access to the randomisation

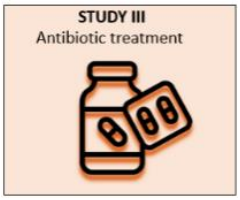
code, sequence or block sizes at any time during the trial. Specimens from the LRT were collected as soon as possible or within 24 hours of admission. The study was an open-label trial. The statistician was blinded until data analysis was completed. Patients in the intervention group had the possibility to deliver one sample (FET or IS) or two samples (FETIS). Participants in the intervention group who could not deliver a sample in the intervention group underwent TS.

Primary outcome: The quality of LRT specimens measured by Gram stain. Samples with < 10 SEC per low power field of view (10 × objective) were classified as good quality (116).

Secondary outcomes: Adverse events included aggravation of vital parameters, mortality within a week and 30-day readmission. Patient experience of the sampling was measured by a five-category Likert-scale from very bad to very good.

Sample size: The study needed 260 patients to reach a power of 84% with a two-sided p-value, and an alpha level of 5%.

Statistics: The primary analysis followed the intention-to-treat protocol and was repeated as a complete case analysis. The primary outcome was analysed using logistic regression. For the secondary outcomes, pooled adverse events and patient experience (Likert-scale), a Poisson regression and Wilcoxon test were performed, respectively.



Study III: The effect of point-of-care multiplex polymerase chain reaction of respiratory specimens on antibiotic treatment of patients acutely admitted with suspected community-acquired pneumonia in Denmark: a multicentre randomized controlled trial

Procedure: LRT specimens were collected right after recruitment. Patients were randomly assigned for the analysis of respiratory samples by i) POC-PCR (the Biofire® FilmArray® Pneumonia Panel plus (160) added to standard care) or ii) SCO (routine culture and, if requested by the attending physician, target-specific PCR). Targets of the Biofire® FilmArray® Pneumonia Panel plus (Biomérieux, Marcy l'Etoile, France) are presented in the supplementary material for paper III. An independent data manager generated the sequence using permuting blocks of varying sizes stratified according to sites. Randomisation was performed by project assistants using a computer-generated randomisation tool from REDCap (158, 159). Allocation concealment was ensured as the project assistants did not have access to the randomisation code, sequence or block sizes at any time during the trial. Patients and investigators owning the data were blinded to the allocation and test results. Outcome adjudicators and clinical staff at the ED were not blinded to allocation and test results but were, together with the statistician, blinded to data management and analysis. Laboratory staff performing standard care analyses was blinded to allocation. The study coordinator was not blinded. After sputum collection and randomisation, the study assistant or laboratory staff immediately performed the POC-PCR analysis in the ED (two sites) or in a laboratory close to the department (transport time less than 10 minutes for one site). Within 4 hours after the

patient was admitted, the result of the POC-PCR was handed to the treating physician along with a guideline-based action card (supplementary material for paper III) recommending specific treatments matching different POC-PCR results. The physician was encouraged to contact the clinical microbiologist for further advice.

Primary outcome: No or narrow antibiotic treatment prescriptions within 4 hours. Defined as antibiotics active against CAP pathogens: beta-lactamase sensitive penicillins (phenoxymethylpenicillin or benzylpenicillin), extended-spectrum beta-lactamase sensitive penicillins (ampicillin/amoxicillin/pivampicillin), and no antibiotics. In the case of penicillin allergy: macrolides and cefuroxime were defined as narrow-spectrum antibiotics (supplementary material for paper III).

Secondary outcomes: No or narrow antibiotic treatment prescriptions within 48 hours and day 5. Targeted and adequate treatment prescriptions within 4 hours, 48 hours and day 5. ICU admissions, 30-day readmission, LOS and mortality (in-hospital and 30-day mortality). Targeted antibiotics were defined as narrow-spectrum antibiotics targeting CAP or antibiotics directed against a detected bacterial pathogen identified by culture. Adequate antibiotics were defined as all antibiotics covering the detected bacterial pathogen (supplementary material for paper III).

Sample size: The calculation yielded a power of 94% with 290 patients with two-sided 5% significance.

Statistics: Intention-to-treat analysis was performed using logistic regression with clustered standard errors to investigate the effect of POC-PCR on antibiotic prescriptions within 4 hours. Furthermore, per-protocol analyses

were performed to investigate antibiotic prescriptions of ‘no or narrow’, ‘targeted’ and ‘adequate’ treatment at all three timelines at 4 hours, 48 hours and day 5. To compare the two groups, negative binomial regression was performed for LOS, the Chi-square test for readmission within 30 days, and Fisher’s exact test for 30 days-mortality, in-hospital mortality and ICU admission.

4.6 Quality monitoring

All studies had a pilot period before the study started and a quality monitoring strategy to support consistent data collection and ensure good quality of internal validity to prevent systematic errors. The steering committee monitored participants’ daily inclusion, and data collection performance was monitored internally. Necessary progress was discussed with the study assistants and steering committee.

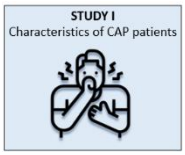
Data cleansing for studies I and III was performed and monitored by at least two project assistants, and the principal investigator checked the files. All project assistants received bedside and simulation training in sampling methods. Standardised protocols were developed for the interventions in both RCT, for FETIS sampling in study II, and for POC-PCR analysis in study III. To prevent performance bias (161) in study II, an external assessor supervised the performance of the project assistants in data collection during sampling methods. An independent microbiology expert assessed the quality of the specimen data in study II. An external product consultant trained and supervised the assistants in managing the FilmArray analysis in study III.

4.7 Ethics

Verbal and written informed consent was obtained from all participants as required by Danish legislation (Appendix C). The processing of personal data was notified to and approved by the Region of Southern Denmark, cf. Art 30 of the EU General Data Protection Regulation, approved by the Regional Committee on Health Research Ethics for Southern Denmark and conducted according to the Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects.

5. SUMMARY OF THE RESULTS

5.1 Study I



Community-acquired pneumonia – use of clinical characteristics of acutely admitted patients in a diagnostic model: a cross-sectional multicentre study

Study I included 954 (43%) of the patients screened for eligibility where the attending physician suspected the patient of having an infection. CAP was suspected in 402 (42%) of the patients. A CAP diagnosis was verified in 265 (28%) recruited patients (Figure 8).

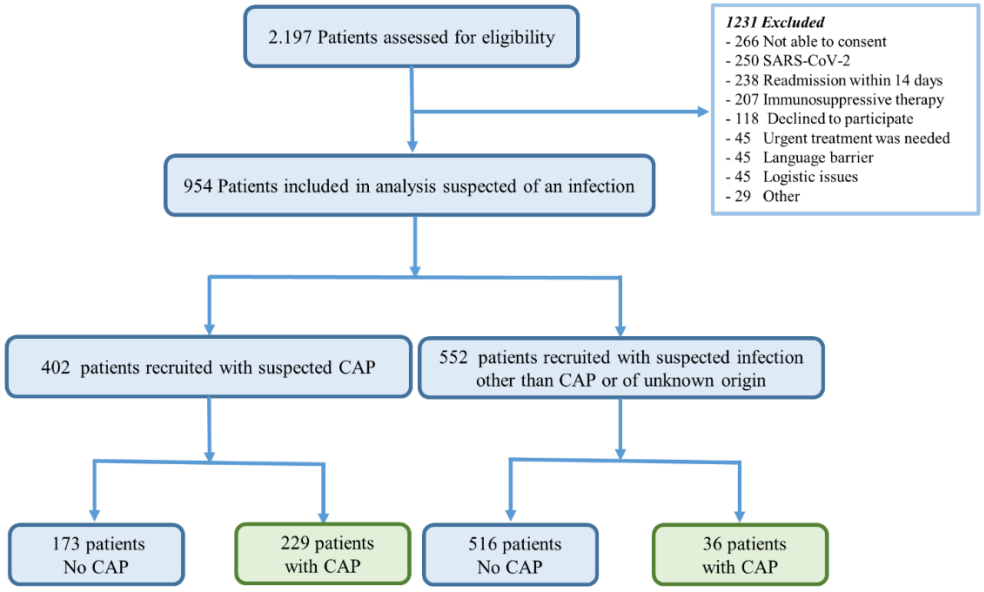


Figure 8: Trial profile

Source: Paper I, Figure 1.

5.1.2 Characteristics of patients with verified community-acquired pneumonia

Of the 954 patients the ED physician suspected of having an infection, patients with verified CAP had a median age of 75 (IQR: 63.5; 82.0) years, 46% were more than 75 years old, and over half were males (54%). There were 27 characteristics identified. The strongest predictors of CAP identified by univariate with a $p < 0.001$ were being a previous smoker, having pulmonary diseases, having previous events of CAP, having the symptoms of a cold, chest pain. Having respiratory symptoms such as dyspnea, cough and expectoration increased the likelihood of CAP more than fourfold. Stethoscope assessment with any abnormal findings from chest auscultation increased the probability of CAP six times. A triage demanding urgent treatment, abnormal vital signs of respiratory rate > 20/min, oxygen saturation < 96 %, and increased leukocytes, neutrophilocytes and CRP values were also predictors for CAP.

Among 402 patients suspected of CAP by the ED physician, 229 (57%) had CAP. Thirteen characteristics were found where gastrointestinal and respiratory symptoms overlapped among patients verified and not verified with CAP. The predictors associated with CAP ($p < 0.001$) were lower oxygen saturation than 96%, sodium (< 137 or > 145 mmol/L), and elevated values of leukocytes and neutrophilocytes. CRP ≥ 100 mg/L increased the likelihood of CAP 11 times compared with those not verified with CAP.

5.1.3 The diagnostic prediction model

The multivariate yielded 13 predictors of CAP: dyspnea, expectoration, cough, common cold, malaise, chest pain, respiratory rate (> 20/min), oxygen saturation (< 96%), abnormal chest auscultation, leucocytes (< 3.5 or > 8.8 10E9/L) and neutrophilocytes (> 7.5 10E9/L). CRP (< 20 mg/L) and having no previous event of CAP contributed negatively to the final model.

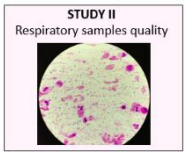
The predictors yielded good prediction performance for CAP with an area under the ROC curve of 85%. Recalibration demonstrated a good prediction of the proportion of CAP patients in the test sample (p = 0.227). Table 6 shows the performance of the predictive model compared with the diagnosis made by the ED physicians.

Table 6: Performance of the predictive model compared with the initial diagnosis made by ED physicians

Performance	Sensitivity % (CI)	Specificity % (CI)	Positive predictive value % (CI)	Negative predictive value % (CI)
Predictive model	86.1 (79.1–93.1)	64.1 (57.1–71.1)	41.6 (34.6–48.6)	93.9 (86.9–100)
Physicians	86.4 (84.2–88.6)	74.9 (72.1–77.6)	57.0 (53.8–60.1)	93.5 (92.0–95.0)

The predictive model had a 35% cut-off and a prevalence of 22%. The prevalence of CAP was 28% in the population of 954 patients suspected of infection.
Source: Paper I, Table 4.

5.2 Study II



Expiratory technique versus tracheal suction to obtain good-quality sputum from patients with suspected lower respiratory tract infection:
a randomized controlled trial

In total, 280 (52.4%) patients were randomised from the 534 patients screened for eligibility. The intention-to-treat population included 141 patients (50.4%) allocated to TS and 139 (49.6%) to FETIS. The complete case analysis comprises 119 (85%) and 67 (48%) patients from the TS and FETIS groups, respectively (Figure 9).

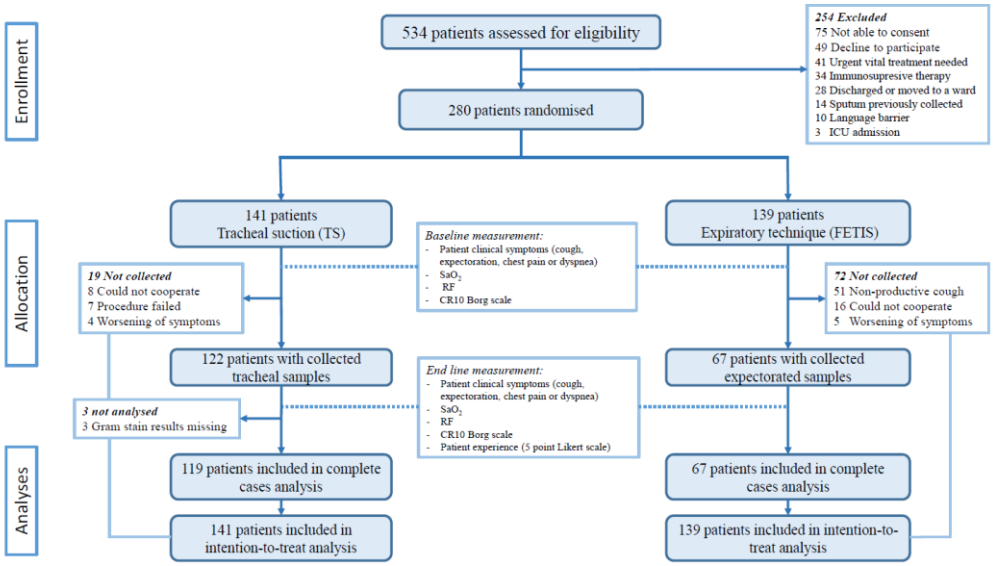


Figure 9: Trial profile
Source: Paper II, Figure I (162).

5.2.1 Quality of respiratory samples

The results of the intention-to-treat analysis showed that the chance of obtaining a good-quality respiratory sample was higher when patients underwent TS compared with FETIS (OR 1.83 [95% CI, 1.05 to 3.19], p = 0.035), and these results were supported by the per-protocol analysis with an OR for TS of 2.42 (95% CI, 1.31 to 4.47), p = 0.005.

5.2.2 Adverse events and patient experience

There was no statistical difference in pooled adverse events between groups. TS pooled adverse events yielded an IRR of 1.21 [95% CI, 0.94 to 1.66]; p = 0.136. Patients allocated to FETIS reported better experiences than those allocated to TS (p < 0.001). Supplementary material for paper II, presents sensitivity analysis for adverse events, harms and subjective reported patient experiences.

5.2.3 Additional results

Results from the primary analysis in study II revealed that TS was the best method for obtaining good-quality respiratory samples. However, to target antibiotic treatment, it is necessary to identify the agent that might cause the infection in the LRT. Therefore, additional unpublished descriptive results for study II were conducted and shown in Table 7, which presents microorganisms yielded from good-quality LRT samples identified by Gram stain and culture stratified by sampling methods.

Table 7: Findings from good-quality respiratory samples stratified by sampling methods

Findings	Sampling method				Total
	TS-IG	FET	IS	TS-SG	
Samples included in the analyses	120	50	58	57	285
Good-quality samples*, total (%)	72 (60)	12 (24)	20 (34)	30 (53)	134 (47)
Gram stain					
Positive samples	13 (18)	6 (50)	8 (40)	6 (20)	33 (25)
All potential pathogens	15 (21)	6 (50)	8 (40)	7 (23)	36 (27)
Gram-positive cocci chains/pairs	6 (8)	2 (17)	2 (10)	2 (7)	12 (9)
Gram-negative diplococci	2 (3)	2 (17)	2 (10)	1 (3)	7 (5)
Gram-positive cocci clusters	1 (2)	0 (0)	0 (0)	0 (0)	1 (<1)
Gram-negative rods	4 (6)	2 (17)	4 (20)	0 (0)	10 (7)
Gram-positive rods	0 (0)	0 (0)	0 (0)	1 (3)	1 (<1)
Gram-positive single	1 (2)	0 (0)	0 (0)	2 (7)	3 (2)
Yeast	1 (2)	0 (0)	0 (0)	1 (3)	2 (1)
Upper airway microbiota	15 (21)	5 (42)	8 (40)	5 (17)	33 (25)
Culture					
Positive samples	30 (42)	7 (58)	13 (65)	15 (50)	65 (48)
All potential pathogens	33 (46)	8 (67)	15 (75)	16 (53)	72 (54)
S.pneumoniae	2 (3)	1 (8)	1 (5)	1 (3)	5 (4)
H.influenzae	0 (0)	1(8)	1 (5)	1(3)	3(2)
M.catarrhalis	1 (2)	2 (17)	1 (5)	2 (7)	6 (4)
S.aureus	10 (14)	1 (8)	0 (0)	3 (10)	14 (10)
Enterobacterales	11 (15)	2 (17)	6 (30)	6 (20)	25 (17)
Yeast	8 (11)	1 (8)	4 (20)	2 (7)	15 (11)
Other**	1 (2)	0 (0)	2 (10)	1 (3)	4 (3)
Upper airway microbiota	9 (7)	2 (17)	1 (5)	2 (7)	14 (10)
No growth of pathogens	30 (42)	3 (25)	4 (20)	13 (43)	50 (37)

Data are presented in numbers and percentages (%). TS-SG: Tracheal secretion from the standard care group, TS-IG: Tracheal secretion from patients in the intervention group that could not deliver a sample, FET: Forced expiratory technique, IS: Induced sputum.

*< 10 squamous epithelial cells per low power field of view

** Other: *P.aeruginosa*, *N.meningitidis*, *Enterococcus sp*. and *S.maltophilia*.

These additional descriptive results included 134 (47%) good-quality samples of all 285 samples collected from the 280 patients in study II. Regardless of the technique used to obtain specimens, few potential pathogens were detected from good-quality samples. The investigation of the Gram stain identified 36 (27%) possible pathogens. The most predominant microorganism identified by Gram stain was Gram-positive cocci chains/pairs (12 (9%)) and Gram-negative rods (10 (7%)). Culture results yielded twice as many microorganisms (72 (54%)) compared with Gram stain results. The most common pathogens of CAP (*S.pneumoniae* and *H.influenzae*) appeared in 8 (6%) samples of good quality. *S.aureus* and *Enterobacterales* were the most detected microorganisms from culture. Culture results detected more *S.aureus* from tracheal secretions, which might indicate contamination from the UAM. Negative samples were more representative in tracheal secretions compared with expiratory technique.

The effects of prior antibiotic treatment on culture results from good-quality samples are shown in Table 8.

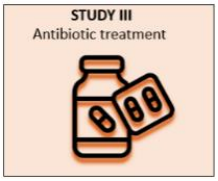
Table 8: Detected pathogens from good-quality respiratory samples from patients treated and not treated with antibiotics within one month before admission

	Antibiotic (NO)	**Antibiotic (YES)**	**Total**
All potential pathogens, total	n = 22	n = 50	n = 72
Culture			
S.pneumoniae	4 (18)	1 (2)	5 (7)
Enterococcus sp.	0 (0)	1 (2)	1 (1)
S.aureus	5 (23)	9 (18)	14 (19)
H.influenzae	0 (0)	3 (6)	3 (4)
Enterobacterales	6 (27)	19 (38)	25 (35)
M.catarrhalis	4 (18)	2 (4)	6 (8)
P.aeruginosa	1 (5)	0 (0)	1 (1)
Yeast	1 (5)	14 (28)	15 (21)
Other	1 (5)	1 (2)	2 (3)

Data are presented in numbers and percentages (%).

From all potential pathogens identified by culture of good-quality samples, 50 (69%) were identified from patients treated with antibiotics within one month before admission. Results showed more *S.pneumoniae* growth in samples from patients not treated with antibiotics, whereas samples from patients treated with antibiotics showed overgrown *Enterobacterales* and yeast (Table 8).

5.3 Study III



The effect of point-of-care multiplex polymerase chain reaction of respiratory specimens on antibiotic treatment of patients acutely admitted with suspected community-acquired pneumonia in Denmark: a multicentre randomized controlled trial

Study III included 294 (77.6%) patients from 379 screened for eligibility. Of the 294 randomised patients, 146 (49.6%) were allocated to SCO and 148 (50.4%) to the POC-PCR group. The analysis of the primary outcome included 291 (99.0%) patients, 145 (49.8%) in the POC-PCR group and 146 (50.2%) in the SCO (Figure 10).

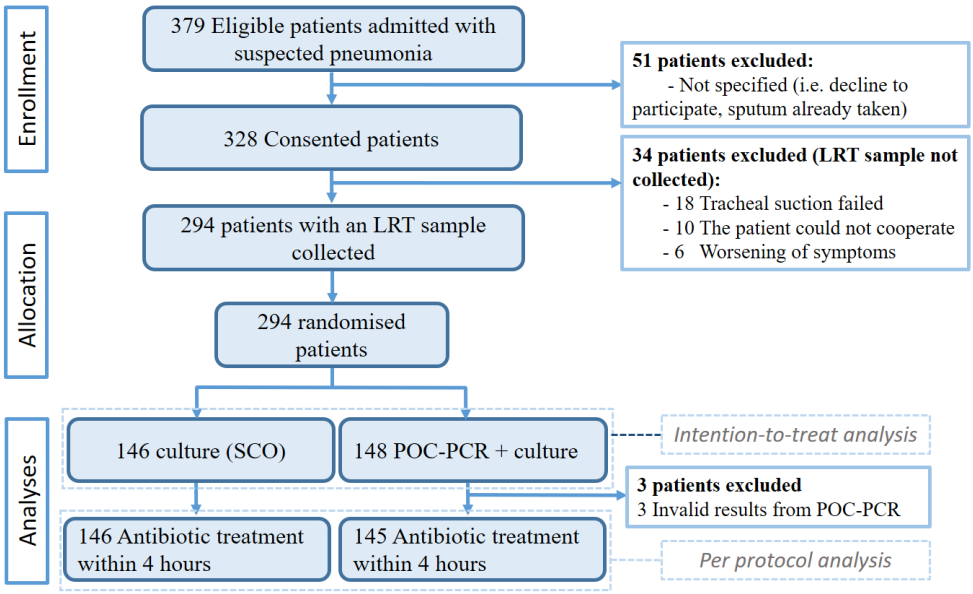


Figure 10: Trial profile
Source: Paper III, Figure 1.

Because of the one death in the POC-PCR group between 48 hours and day 5, the analyses of day 5 had one less patient included. Given the observed difference in triage between the intervention and control, the thesis will present adjusted results. Unadjusted results are presented in paper III.

5.3.1 No or narrow antibiotic treatment

POC-PCR was not superior to SCO regarding prescriptions of no or narrow-spectrum antibiotics within 4 hours after admission. Intention-to-treat analyses of 294 patients yielded an OR of 1.13 [95% CI, 0.96 to 1.34]; p = 0.134, and per-protocol analysis of 291 patients resulted in an OR of 1.14 [95% CI, 0.97 to 1.34]; p = 0.101. This difference decreases at 48 hours, where the chance for patients to receive no or narrow antibiotics was almost equal between groups (OR 0.91 [95% CI, 0.82 to 1.00], p = 0.065). On day 5, patients in the SCO group were more likely to receive no or narrow antibiotics (OR 0.81 [95% CI, 0.72 to 0.91], p = 0.001).

5.3.2 Targeted and Adequate treatment

Analyses of targeted and adequate treatment were based on 55 positive culture results from 290 patients. Results from targeted antibiotic treatment at 4 hours (OR 5.68 [95% CI, 2.49 to 12.94], p = 0.000) and 48 hours (OR 4.20 [95% CI, 1.87 to 9.40], p = 0.000) and adequate antibiotic treatment from 48 hours (OR 2.11 [95% CI, 1.23 to 3.61], p = 0.006) and day 5 (OR 1.40 [95% CI, 1.18 to 1.66], p = 0.000) presented statistically significant differences indicating that patients in the POC-PCR group received earlier targeted and more adequate treatment than patients in the SCO group.

5.3.3 Adverse events

There were no statistically significant differences between POC-PCR and SCO regarding patient 30-day mortality (OR 1.24 [95% CI 0.32 to 4.82], p = 0.749), in-hospital mortality (OR 0.98 [95% CI, 0.19 to 5.06], p = 0.986), admission to ICU (OR 0.54 [95% CI, 0.10 to 2.91], p = 0.475), 30-day readmission (OR 0.90 [95% CI, 0.43 to 1.86], p = 0.787) and LOS IRR 0.82 (0.63;1.07), p = 0.164. This reduction in LOS corresponds to almost one day less in hospital for patients allocated to POC-PCR.

5.3.4 Additional results

Additional unpublished results were conducted to investigate the findings from POC-PCR and cultures among the two groups (Table 9). POC-PCR detected 187 possible pathogens (bacteria and viruses) in 109 (58%) positive samples. A single potential pathogen was identified in 54 samples, and two or more possible pathogens were detected in 55 samples. Viruses comprise 35 (24%) of the possible pathogens identified. Compared with POC-PCR, culture identified five times fewer microorganisms from the same population, with a total of 32 (22%) potential pathogens. A single microorganism was identified in 22 (15%) of the samples, and 5 (3%) identified two microorganisms. Similar results were found from patients allocated to SCO, where culture identified 36 (25%) microorganisms, 32 (22%) with a single and 2 (1%) with two microorganisms.

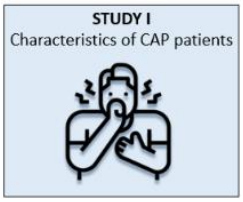
Table 9: Microbial aetiology from POC-PCR and cultures
POC-PCR† alone (n=145), culture from samples of the POC-PCR group (n=145), and culture from the standard care group (n=SCO††) (146).

Microbial aetiology	Analyses methods			Total
Total, n	Group POC-PCR		Group SCO	
	POC-PCR n = 145	Culture n = 145	Culture n = 146	n = 291
Bacteria				
S.pneumoniae	16 (11)	9 (6)	7 (5)	32 (11)
H.influenzae	61 (42)	16 (11)	6 (4)	83 (28)
M.catarrhalis	16 (11)	1 (< 1)	5 (3)	22 (8)
P.aeruginosa	3 (2)	1 (< 1)	0 (0)	4 (1.5)
S.aureus	30 (21)	2 (1)	9 (6)	41 (14)
*Enterobacterales**	19 (13)	2 (1)	6 (4)	27 (9)
L.pneumophila	0	1 (< 1)**	0	1 (< 1)
Others***	7 (5)	-	3 (2)	10 (3)
Total Bacteria	**152 (105)**	**32 (22)**	**36 (25)**	**220 (76)**
Viruses				
H.Rhinovirus/enterovirus	14 (10)	-	1(< 1)**	15 (5)
Corona	7 (5)	-	-	7 (2.5)
Parainfluenza	5 (3)	-	-	5 (2)
Respiratory syncytial	4 (3)	-	-	4 (1.5)
H.metapneumovirus	3 (2)	-	-	3 (1)
Influenza A	2 (1)	-	-	2 (< 1)
SARS-CoV-2	-	3 (2)**	-	3 (1)
Total viruses****	**35 (24)**	**3 (2)**	**1(< 1)**	**39 (13)**

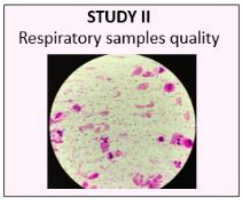
Data are presented in numbers and percentages (%). † POC-PCR: Point-of-care polymerase chain reaction †† SCO: Standard care only
*Enterobacterales group from POC-PCR: *E.cloacae, E.coli, K.aerogenes, K.oxytoca, K.pneumoniae* group, *Proteus spp. S.marcescens*. *Enterobacterales* group from culture: *Enterobacterales, Klebsiella*.
**Routine PCR.
***Others: *A.baumannii complex, S.agalactiae,* and *Hemolytic streptococcus*.
**** 35 viruses from 33 samples, viruses from routine PCR-assay were not registered. No findings of: *C.pneumoniae, M.pneumoniae, S.pyogenes*, Influenza B., MERS-CoV.

6. DISCUSSION

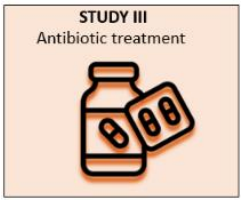
6.1 Key findings



- The prevalence of CAP during this study period (1 March 2021 to 28 February 2022) was 28%, and approximately half of the patients were ≥ 75 years old and male.
- Verified and not verified CAP presented with a range of overlapping symptoms.
- Among the 954 patients with suspected infection, cough, expectoration, abnormal findings from stethoscope assessment and CRP ≥ 100 mg/L were strong independent predictors that increased the likelihood of a verified CAP diagnosis fourfold.
- Among 402 patients with a suspected CAP diagnosis, 57% had the diagnosis verified. CRP ≥ 100 mg/L stands out as a predictor increasing the likelihood of a CAP diagnosis more than 11 times.
- Thirteen recognised predictors of CAP ended in the final diagnostic model. This model reached a good AUC performance of 0.85 (CI: 0.77–0.92) and calibration (p = 0.227), and with the best cut-off of 0.35, the model yielded an 86% sensitivity and 64% specificity. However, ED physicians diagnosed more accurately and successfully than the model.



- TS had nearly twice the likelihood of ensuring a good-quality specimen than FETIS.
- There was no difference between TS and FETIS in pooled adverse events.
- Patients allocated to the FETIS group had more positive experiences than patients allocated to TS.
- Despite efforts to improve sampling collection, only half of the patients allocated to the FETIS group could deliver a sample and less than half of these samples were of good quality.
- Gram stain and culture results of good-quality samples detected few possible pathogens.
- Antibiotic treatment affects culture results from good-quality samples, with results identifying fewer potential pathogens and the overgrowing of opportunistic pathogens.



- There was no difference in the prescriptions of no or narrow antibiotics between the POC-PCR and SCO groups.
- Results indicated that patients allocated to POC-PCR might receive earlier and more targeted and adequate treatment.
- There was no statistical difference in mortality (in-hospital or within 30 days), ICU admissions, readmissions within 30 days or LOS. However,

patients allocated to POC-PCR had almost one day less in the hospital compared with SCO.

- POC-PCR detected considerably more possible pathogens, including 24% identified viruses.

6.2 Comparison of key findings with existing literature

6.2.1 Clinical characteristics of community-acquired pneumonia

Many recent studies have described clinical variety in the manifestation of CAP, such as fever, cough, increased respiratory frequency, increased heart rate, expectoration, dyspnea, chest pain and crackles from auscultation (26, 59, 62, 67, 163). CAP patients presented with some of the same characteristics from the time of Hippocrates (1) to today. The diagnostic model discussed in this thesis identified the same features, even with a broader range of candidate predictors and modern analysis methods for a more accurate prediction. Some studies reported atypical symptoms of CAP, such as headache, delirium, vomiting, loss of appetite, abdominal pain indicative of appendicitis or complete absence of any respiratory symptoms, particularly in the elderly (59, 68, 70). This was not the case in this study, even though all these factors were included in the multivariate analysis. However, this study did identify single atypical symptoms and signs of CAP when patients with suspected infection or suspected CAP were compared. In agreement with other studies, symptoms overlapped, providing a challenge for ED physicians to distinguish CAP from other conditions, especially if the suspected diagnosis was CAP (26, 130). A recent systematic review reported that nonspecific symptoms could lead to misdiagnosis-related harms, especially in the initial assessment by physicians, and could occur in 54% of the cases in EDs (33). The authors

found a similar degree of diagnostic uncertainty (sensitivity 0.86 [0.80–0.90], specificity 0.76 [0.71–0.80], positive predictive value 0.66 [0.59–0.71] and negative predictive value 0.91 [0.87–0.93]) (33) when compared with the study outlined in this thesis. The question is how much uncertainty would be allowed in the diagnosis of CAP. As Kassirer noted in 1989:

> *Absolute certainty in diagnosis is unattainable, no matter how much information we gather, how many observations we make, or how many tests we perform … Our task is not to attain certainty, but rather to reduce the level of diagnostic uncertainty enough to make optimal therapeutic decisions* (32).

Considering the biomarkers investigated, other studies reported results similar to this thesis (64, 80, 163, 164). Elevated values of CRP stand out as a strong predictor regardless of the CAP population studied – for instance, characteristics of patients suspected of infection, suspected of CAP, and as a result from the diagnostic model. A diagnostic study investigating a clinical model for CAP included the symptoms of fever, cough, sputum production, abnormal chest auscultation and dyspnea and yielded ROC AUC of 0.79 [0.75–0.83]. The inclusion of PCT combined with high sensitive CRP in the model resulted in a higher ROC AUC of 0.92 [0.89–0.94] (65). It indicated that the accuracy in diagnosing CAP could be improved by incorporating other biomarkers.

The diagnostic prediction model outlined in this thesis had a good performance and calibration, as reported by others (165-167). However, because the ED physicians demonstrated a more accurate diagnosis of CAP, the diagnostic model could be interpreted as of limited value to guide a presumptive CAP diagnosis in the specified setting. Nevertheless, the

prevalence of CAP differed between the model (22%) and the population as determined by the expert panel (28%) which may influence the performance of the predictive model (Table 4, study I). Furthermore, an external validation would reveal the model's usefulness and value in EDs organised differently or in settings with fewer resources or high workflow where other healthcare professionals could help screen patients.

This thesis confirms the clinical characteristics observed over decades when these results are compared with the literature, such as fever, cough, expectoration and dyspnea. There are several characteristic symptoms and signs of CAP, some of which are strong predictors. Care should be taken labelling 'atypical symptoms' as often they have been recognised in the past, however, including nausea, loss of appetite and malaise (1). Consideration should be given to a more heterogeneous CAP diagnosis, and the outdated diagnostic setup also needs examination. There is room for improvement in the diagnosis of CAP, but it needs a renewed emphasis on combining traditional clinical skills with new diagnostic tools. In an era of rapidly developing technology, new diagnostic tools such as biomarkers, PCR or imaging should be incorporated into the diagnostic process in a balanced manner. As suggested in Figure 2 in the introduction, the inclusion of these tools should assist rather than substitute for clinical reasoning in facilitating an accurate and timely diagnosis (32).

6.2.2 Respiratory samples quality

Almost 20 years ago, routine Gram staining and culture of expectorated sputum was labelled as the 'sacred cow' and critically debated among specialists in the field as

> *a hallowed, time-honored tradition of dubious value. The overall recent trend has been, finally, to relegate this sacred cow of a test to the quaint pastures of history, but some steadfastly cling, with purple-stained fingers, to the hope that expectorated sputum analysis, as it is currently applied, can somehow reliably improve clinical decisions when managing patients with community-acquired pneumonia* (168).

This comment appeared after the presentation of results from a study including 1669 patients with CAP before antibiotics were administered at the ED (112). The results shown in this thesis are similar, as expectorated sputum in the FETIS group could only be obtained from half of the patients despite efforts using expiratory techniques and saline inhalation to induce the sputum. These efforts have been reported to improve expectoration in some populations (106, 111, 169-172). As outlined in other published studies, this thesis found that under half of these samples were of good quality, leaving few detected potential pathogens to guide ED physicians in adjusting the antibiotic therapy (112, 128, 129, 173, 174).

Patients allocated to TS had substantially more samples collected (87%), and the quality was almost twice as better as FETIS. From a patient perspective, compared with patients in the FETIS group, TS was more invasive, resulting in mild adverse events such as procedure-related bleeding and aggravation of dyspnea, but no difference was found between groups when adverse events were pooled. Other studies have reported some adverse events from IS, such

as bronchospasms (109, 175, 176). In the study reported in this thesis, isotonic saline inhalation and lower duration (10 min) was chosen, which may have contributed to fewer adverse events and a relatively high number of patients describing the procedure as 'good'. Other studies reported more collected samples and higher microbial yield from subgroups of patients with *Mycobacterium tuberculosis* and *Pneumocystis jiroveci* pneumonia using hypertonic saline inhalation (176-179). However, in this study a saline dose primarily used in clinical practice in Denmark and well-tolerated by patients was prioritised.

With a high percentage of successfully collected samples of good quality and the acceptability of the procedure from patients, implementing TS in the ED could solve some challenges regarding the collection and analysis of LRT samples. Therefore, the question regarding the utility of LRT samples as a diagnostic tool and their clinical effects with regard to guided antibiotic treatment decisions remains relevant. Two recent systematic reviews and meta-analyses (104, 122) showed that a positive sputum Gram stain from good-quality sputa could identify the causative pathogen of CAP, confirming the diagnosis and leading to appropriate antibiotic choice. Studies reported high specificity and sensitivity for the most common CAP pathogens (sensitivity and specificity of 0.69, 0.91 and 0.76, 0.97 for *S.pneumoniae*, and *H.influenzae*, respectively) (104). However, the major challenge is that even when many good-quality samples were collected, the microbial yield in this study was low, with a total of 6% for *S.pneumoniae* and *H.influenzae* when combined. Therefore, only a few patients would need a revision of the antibiotic treatment, and there is a risk of not adjusting the treatment to the correct pathogen. Pathogens might remain unidentified after a Gram stain because of their limited size and amount, increasing the risk of treatment

failure. This could be through a resistant bacterial agent receiving just penicillin or, on the contrary, an identified Gram-negative microorganism treated with broad-spectrum antibiotics even if the causative pathogen was *S.pneumoniae*. It has been reported that the false-negative proportion for Gram stain investigation was 44% for *S.pneumoniae* and 22% for *H.influenzae,* suggesting that stopping antimicrobials after a negative sputum Gram stain result in patients may not be appropriate (122). That is, failure to detect these causative pathogens by Gram stain investigation does not conclusively show their absence.

As stated in other studies and indicated in the additional results reported in this thesis, the sensitivity of Gram stain and culture decreased, especially when patients were treated with antibiotics prior to admission (104, 127). Therefore, patients may be at risk of receiving inappropriate antibiotics because of overgrown Gram-negative microorganisms, making it difficult to argue for the use of these specimens in clinical practice if antibiotics have been administered before admission.

6.2.3 Point-of-care polymerase chain reaction - Solution for rational use of antibiotics?

To solve the problem of long turnaround times for test results and low test sensitivity, and to increase the possibility of a targeted treatment, the study reported in this thesis tested the effect of adding POC-PCR to standard care in relation to antibiotic treatment. There was no difference between POC-PCR and SCO in the prescriptions of no or narrow-spectrum antibiotics at 4 hours and 48 hours. On day 5, a statistically significant difference was found. However, it is difficult to consider this minor difference for this secondary outcome clinically significant. There are several explanations for the negative

result concerning the primary outcome. Denmark has low AMR, where most pneumococci and *H.influenzae* are susceptible to penicillins (147), and the study's setting already has a prudent, considered use of empirical antibiotics. The results of no or narrow antibiotic prescriptions could have yielded positive results in settings with a higher rate of antibiotic prescriptions.

Furthermore, the timing of this study coincided with the SARS-CoV-2 pandemic, when the prevalence of viruses was generally very low (180). It is known from the additional results that viruses comprised 13% of the pathogens identified in the study's population of patients suspected of CAP (2.7% isolated virus detection), while other studies reported viruses in 20%–40 % of CAP cases (59, 132, 135). However, PCR for viruses could be prescribed on demand for patients in the SCO group, and more viruses may have been detected if all patients had been tested. In periods with a higher transmission of respiratory viruses, POC-PCR could potentially reduce the use of antibiotics as patients with virus aetiologies are managed differently. Another explanation is that adhering to the recommendations of the action card according to the PCR results may have broadened the antibiotic therapy unnecessarily. As expected, POC-PCR yielded substantially more microorganisms than culture, almost twice the rate for *S.pneumoniae* (11% v. 6%) and nearly four times more for *H.influenzae* (42% v. 11%). There was also a significant difference identified from POC-PCR compared with culture regarding *S.aureus* (21% v. 1%), *Enterobacterales* (13% v. 1%) and *M.catarralis* (11% v. < 1%). The incidence of *Enterobacterales* as causative of CAP is reported to be 1.3% (181), and they are likely overgrown from patients pretreated with antibiotics (127, 129). Therefore, *Enterobacterales* was described as a 'very rare causative pathogen' in the action card reported in this thesis. *Enterobacterales* and *S.aureus* generally represent

contamination of the upper airway microbiota in CAP (100, 181). Individuals with CAP caused by these agents may present as more severely ill and with comorbidities. Therefore, patient history and clinical features are essential in cases where the antibiotic treatment must be broader (181). In this study, the most severely ill patients were excluded, and one-quarter of the patients received antibiotics before admission. This means that findings of *Enterobacterales* and *S.aureus* from POC-PCR are unlikely to be pathogens and may be false positives, the same suggested by (100, 136).

Half of the samples analysed by POC-PCR had the occurrence of more than one microorganism. For co-findings, the guideline-based action card suggested treatment for the most resistant pathogen. For instance, in case of detection of *M.catarralis* and *S.pneumoniae,* the recommendation would be treatment with piperacillin-tazobactam or amoxicillin-clavulanic as *M.catarralis* is not common but a potential pathogen of CAP, especially in patients with chronic pulmonary diseases (182). However, *M.catarralis* may originate from contamination of the upper airway microbiota; in this case, the patient would be overtreated with antibiotics. The benefit of POC-PCR would be the rapid treatment of those patients to prevent deterioration. However, the risk of targeting the incorrect pathogen is one of the concerns of sensitive molecular methods. These methods cannot differentiate pathogens from contamination. The risk of such treatment failure is considered an error in the diagnostic process, contributing to a greater risk for harm than ensuring diagnostic certainty (32).

To overcome this problem almost 80% of the patients had respiratory samples collected by TS. Nevertheless, for the benefit of POC-PCR in the diagnostic process, results must be considered together with patients' clinical

presentation, disease severity, comorbidities, risk factors and cross-professional consultation in the diagnostic team (e.g., nurses, ED physicians, microbiologists and others).

Interestingly, based on culture results, patients in the POC-PCR group received earlier and more targeted and adequate antibiotics within the first two days. Even though these analyses were done using a small subsample, and it is unknown if the results could be generalised to the rest of the study population, this indicates that POC-PCR might contribute to a more targeted treatment without using unnecessarily broad-spectrum antibiotics. Applying the results from study I, if POC-PCR were performed, 86% of the patients would have received the possibility of a targeted CAP therapy, and 57% would potentially end with a targeted treatment. For patients where CAP could not be confirmed, the information would enable the ED physician to rethink the working diagnosis early.

Another interesting observation from the POC-PCR group was the reduction in LOS of almost one day. Although not statistically significant, this may be clinically and economically significant, considering that patients admitted to the medical speciality have a mean hospital LOS of 5.9 days (183). Local data from settings in the study period showed a mean hospital LOS of 3.8 days for adult patients discharged with a pneumonia diagnosis.

6.3 Methodological considerations

6.3.1 Methodological considerations, study I

Another model could have been used to build the diagnostic prediction model. Nonetheless, LASSO has an advantage with a range of variables and infliction of a penalty if the model leans towards overfitting. Instead of shrinking the number of variables using LASSO, an alternative could have been stepwise logistic regression modelling, where strong independent predictors from the univariate logistic model could be included in a final model and variable selection is based on a variety of statistical significance tests. This would have allowed the selection of predictors step by step. However, the explorative approach using LASSO enabled variables to be tested as continuous, dichotomous and categorical, including and excluding them in the model with different combinations to ensure the optimal model. Furthermore, LASSO has advantages regarding testing on extern data whereas stepwise is more sensitive to own data / training data.

Unfortunately, there is no gold standard for diagnosing CAP. The routinely used diagnostic tools such as CXR and CRP are unspecific. Therefore, the reference standard for CAP diagnosis was assessment by an expert panel. Despite considerable experience as emergency and infectious diseases specialists with considerable experience, they were still physicians evaluating other colleagues' judgement of CAP. Specialists of the expert panel might have a better prerequisite to diagnose CAP in suspected CAP patients because of the availability of results from imaging (HRCT, ULDCT and CXR), microbiological tests (POC-PCR and culture) and improved registration of patients' symptoms. Therefore, differential verification bias might occur, overestimating the ED physician's accuracy in diagnosing CAP (184).

Furthermore, the questions asked by the project assistants and ED physicians have similarities as both were based on patient anamnesis and history, which might contribute to the diagnostic model performance being closely comparable to the initial diagnosis made by the ED physicians.

6.3.2 Methodological considerations, study II

An alternative study design was considered with regard to comparing respiratory samples' quality. Collecting two or more specimens from the same patient could enable the comparison of sample quality and microorganisms within the same patient. In this case, another sample type should be included instead of expectorated sputum, as only half of the patients could expectorate, which would still entail a significant number of missing data. Nasopharyngeal, oropharyngeal, BAL and mini-BAL were discussed among microbiologists at the study's sites. Comparison of these specimens could also have contributed to the evidence gap in the investigation of specimen quality from patients acutely admitted with LRTI. Nasopharyngeal and oropharyngeal are questionable in identifying bacterial pathogens from the LRT but have advantages in identifying respiratory viruses in patients with suspected LRTI and are non-invasive (185-187). BAL and mini-BAL can detect significantly more LRT bacterial pathogens than nasotracheal suctioning and expectorated sputum but are invasive and require more resources (188, 189). However, expectorated sputum was chosen as it is the most commonly used method in Denmark and widely used in research on patients acutely admitted with CAP (104, 112). As many patients have difficulty expectorating, saline inhalation to induce the sputum and facilitate expectoration was added (108, 109). TS was the standard care technique described in the guidelines as the preferred method because of minimal contamination from the upper airway. Moreover, TS was already routinely

used at one of the sites where most patients were included (15, 16, 189). Therefore, a comparison of these two methods was deemed most relevant. The supervision of nurses revealed that TS was the less desirable procedure as nursing staff were afraid of harming patients. TS is more invasive than FETIS, and nurses' motivation for patient recruitment for the project was to show that FETIS was not worse than TS in obtaining good-quality samples. Therefore, a randomised controlled trial (RCT) testing the non-inferiority of FETIS was considered a relevant design instead of a superiority trial.

Other considerations regarding taking several samples from the same patient and comparing them were that the diagnostic yield might decrease and that, depending on the choice of the sampling method, exposing patients to several techniques was ethically challenging. However, it could have been interesting to collect sputa from those able to expectorate purulent sputum – judged macroscopically following the colour classification proposed by Murray and Washington (190) – and TS from those who failed. Although the groups would not be equally distributed with an RCT, the results may be more readily implementable, especially if the quality of sputum was comparable.

6.3.3 Methodological considerations, study III

Study III tested superiority and concluded that POC-PCR was not superior to SCO for the prescription of no or narrow-spectrum antibiotics treatment. A non-inferiority randomized controlled trial (RCT) examining the hypothesis that POC-PCR would demonstrate non-inferiority to SCO in terms of no or narrow-spectrum antibiotic prescriptions may have yielded a positive outcome. This could be attributed to POC-PCR's ability to detect viruses and common pathogens associated with CAP, allowing patients to be appropriately treated with narrower-spectrum antibiotics or even without

antibiotics following the restrictive antiobiotic guidelines in Danish EDs. However, such a trial but would require a larger sample size (191).

POC-PCR analysis was performed in many cases before blood tests or imaging results became available, ensuring a quick contribution to the diagnostic process. However, it could be interesting, and the test might be more relevant, timely and efficient, if POC-PCR analysis was added to the results of other diagnostic tools to ensure a targeted treatment. Information like this may decrease diagnostic and treatment errors without any meaningful time delay (32).

Intention-to-treat analyses are commonly performed as the primary analysis in confirmatory trials (192). There were three missing samples caused by POC-PCR assay failure. Thus, no clinical characteristics influenced the missing mechanism. This suggests that the data were missing completely at random, and imputation was deemed unnecessary under the acceptance of minimal loss of power (193). Furthermore, the number of missing data was negligible, also indicating that imputation would not be needed (194). For these reasons, intention-to-treat and per-protocol analyses were expected to yield the same results, as they did. However, based on the superiority design, for sensitivity reasons, multiple imputation was performed.

The standard design of randomising patients at admission before POC-PCR was used, which offered the effectiveness of POC-PCR as an aid to the management CAP patients as planned. Another strategy would be to perform a random disclosure design where all patients suspected of CAP have LRT specimens analysed by POC-PCR before 1:1 randomisation is performed. This would involve the establishment of a disclosure group where patients receive treatment-targeted POC-PCR results and a non-disclosure group

where POC-PCR results are unavailable to clinicians or patients and patients are treated empirically (standard care) (195, 196). If designed with enough power, a subgroup analysis could be performed to investigate the effectiveness separately in test-positive and test-negative patients leading to evidence-practice recommendations. If treatment response is better in the POC-PCR group for test-positive patients but not test-negative patients, this could be a rationale for implementing the test in clinical practice. Another strategy could be to only randomise the patients with positive test results, where only the results for patients randomised to POC-PCR would be available to clinicians (197). These designs would require more resources and may have ethical issues, but the advantages of these strategies are the ability to compare the two tests and, in addition, test the prognostic accuracy of the effects of POC-PCR on patient-reported outcomes, adding more value to the results. As stated in 1978:

> *Diagnosis is not an end in itself. Physicians perform tests on patients to gain information about the presence or absence of disease (screening and diagnosis), to help plan treatment in cases where disease is established, and to monitor the results of treatment. The effect we value in its own right is the health of patients, both the length and quality of their lives, including peace of mind. In general medicine is directed toward the goal of improved health outcomes* (198).

The next step for study III would be to perform secondary analyses focusing on the POC-PCR group where the same patient has findings from POC-PCR and culture. These analyses would be interesting and relevant to investigate diagnostic accuracy and to understand the effects of antibiotic treatment and

the association of the copies/mL from the POC-PCR compared with pathogens' findings from culture.

6.4 Strengths

A major strength was having dedicated project assistants connected only to the three projects. All were involved in the selection of variables to be studied and discussed how standardised data collection should be achieved. All had standardised written protocols for the interventions and contributed to quality monitoring, such as double-checking the database and having case discussions during the recruitment and inclusion of patients. These processes may have contributed to the study's internal validity. Also considered was how data could be collected by nurses or other personnel during routine shifts and implemented in the ED workflow, which would be convenient and probably be more economically affordable. However, this was not implemented as it would be challenging to collect data within the study period and be engaged in a project during busy shifts and emergency tasks, especially during the pandemic.

From my point of view, collaborating as part of a research group for the INDEED project was a strength, not only regarding practical concerns such as faster data collection and collaborating with regard to data register and curation, but also enriching academic and professional development with multifaceted professional discussions. It also benefited higher project quality with discussion from several perspectives informing implementation, and in addition, the validity of the results was discussed in detail before any submissions and conferences.

Another strength concerns the prospective, multicentre and embedded RCT designs. The study was planned specifically to answer the study questions of

this thesis, including variables prospectively collected to study the prevalence and predictors of CAP. The cross-sectional design enabled exploration of other infections as part of the INDEED project (199). The multicentre design ensured quicker recruitment, diverse population coverage and a higher chance for generalisability and external validity (200). Investigating causality with minimal bias and confounding factors is the main advantage of an RCT design, where the effectiveness of sampling and microbiological analysis methods could be tested (161).

Finally, the key to successfully running and completing such studies in crowded EDs is the indispensable cooperation and collaboration among partners across specialties, well-organised EDs, simply designed RCTs and chief investigators with in-depth insight into an ED's workflow and management ensuring realistic project setup.

6.5 Limitations

6.5.1. Selection of patients

Selecting a study population during the SARS-CoV-2 pandemic is a limitation of the three studies. The citizens followed many restrictions and recommendations, such as social isolation, increased hygiene interventions and vaccination policies that reduced virus transmission and bacterial LRTI (147). This may have interfered with the generalisability of the results to non-pandemic periods, especially concerning the detection of pathogens. Because of organisational factors, the population was limited to patients admitted to a medical ED. Consequently, some patients with CAP and other primary diagnoses at admission might not have been assessed for eligibility and were not included in the studies (e.g., gastrointestinal pain, hip fracture and cardiac heart failure). Furthermore, because the inclusion of patients often occurred

directly after a clinical assessment but before diagnostic tests, some patients not suspected of infection at this time were not eligible and therefore not included. Patients were not included if the presumptive diagnosis have been revised within few hours after blood test results and CXR – for instance, a patient was not suspected of having an infection but after blood tests the clinician suspected CAP. In addition, the population was restricted to patients with mild or moderate CAP, with only 13% with a CURB-65 ≥ 3. In addition, recruitment during daytime and weekdays only might exclude those with severe conditions or acute cognitive impairment who could not consent, excluding patients with a high risk of ICU admission and mortality (201).

6.5.2 Disadvantages of a multicentre design

Although there are many benefits from running a multicentre study, some procedures cannot be precisely the same because of organisational and cultural differences (200). For example, the PCR tool was situated differently in all three sites but in an acceptable margin to be called POC. TS was implemented well at one, and some patients suspected of infection prior to admission had CXR done before the clinical assessment at one site. For more uniform procedures, an implementation strategy should be considered before running the studies. However, it would be waste of resources to implement procedures and stop again in case of negative results. Statistical analysis can account for variances between sites in general. However, because it does not account for individual contextual variation in the different settings, analyses are not reproducible (200).

6.5.3 Patient and public involvement

Clinical research benefits from patient and public involvement (PPI), and studies should be managed in close collaboration with patients and public

participation (202). To some degree, patients were involved in the planning of study II, where two focus group interviews were conducted on the data of sampling methods, bedside questions about symptoms aggravation, Borg scale and patient sampling method experience. Unfortunately, this process has not been reported, and the results of the focus group interviews have not been described in the protocol or other supplementary materials. The lack of PPI is a limitation of all three studies, where multiple perspectives from patients, clinicians and decision-makers could have been explored and incorporated into the research. An example of this could be involving nurses and patients in the choice of sampling methods or patients in the methodology of the POC-PCR study, specifically whether they would like to know the results and be involved in the treatment. Alternatively, the inclusion of the ED physicians' thoughts regarding POC-PCR results and adherence to the guideline-based action card would benefit collaboration during the study. Nevertheless, a PPI is more appropriate now, as results are available and the barriers implementing TS or POC-PCR in clinical practice need to be understood.

Furthermore, transparency in publishing a PPI can contribute to further research. Studies have reported a range of barriers for physicians that choose not to prescribe narrow-spectrum antibiotics (28, 203). This knowledge could be used to investigate if these barriers were the same in this study, which could contribute to low compliance in following the action card, influencing the results. ED physicians' adherence to the guideline-based action was also not measured and is considered a limitation in study III. Finally, the steering committee for this study involved several experts in different fields, given they were 'users' with knowledge of the needs in clinical practice. However, an external panel with broad perspectives from patients, nurses and ED

physicians could have contributed to the steering committee at different stages through an involvement strategy.

6.5.4 Reporting randomised controlled trials

Reporting all amendments and processes in all stages is essential for clinical trials and is seen as a quality seal. Well-conducted but poorly reported trials are misclassified (204). To strengthen the validity and interpretation of the results in open-label trials as this, and to be transparent in the trial report, a blinded interpretation (205, 206) could be performed and uploaded at the first author's university site, 'Pure'. This would increase the transparency of the process with a neutral reporting of the results. In 'Pure', reports, amendments and protocols can be uploaded to be available for other researchers and reviewers.

6.6 Generalisability

As has been discussed, the characteristics of patients with suspect CAP have been the same for many years. The diagnostic prediction model developed in this study might be applicable to other settings after external testing. The limitation would be to have a technology that can calculate the CAP-score efficiently, extracting the values from the patient medical record. TS is a simple method that can ensure good-quality samples and can be performed in settings with minimal requirements. This study showed that onsite POC-PCR could be implemented in EDs in Denmark and related countries but might only be generalisable to well-resourced settings with similar organisations, appropriately trained staff and a rapid POC-PCR service.

6.7 Reflections and implication for clinical practice

What would be the recommendations for the diagnosis of CAP based only on clinical presentation upon arrival at the ED?

ED physicians are already aware of the need to integrate the strong predictors of CAP identified in this model in their clinical reasoning. An accurate history is central to the diagnostic process facilitating an efficient physical exam and contributing to the utilisation of appropriate diagnostic testing. As William Osler has said: 'Just listen to your patient, he is telling you the diagnosis' (32). However, failure in clinical reasoning from inadequate bedside anamnesis is one of the major reasons for diagnostic errors in EDs (33). Therefore, ED physicians must interpret the anamnesis results in conjunction with diagnostic tests and close collaboration with the diagnostic team to assist in precise CAP diagnosis.

What would be the recommendations for collecting LRT specimens?

If the results of LRT specimens' quality are examined comparing TS and FETIS, the recommendation would be TS. However, even with samples of good quality, the microbiological yield for diagnostic purposes is very low. It is difficult to imagine these tests clinically affecting patient outcomes. Therefore, recommendations might not be a 'one size fits all', such as routine and uncritical recommendation of TS for patients suspected of LRTI, but rather a restricted recommendation for subgroups and patients not receiving antibiotics at admission. Furthermore, consistent LRT specimen collection might contribute to the inappropriate allocation of resources. Nonetheless, based on the study's limitations and the fact that patient outcomes were not evaluated other than mortality and readmission as secondary endpoints, abolishing this test cannot be recommended. Regardless of the recommendation, it is essential to have an implementation strategy so that the professionals performing tests feel competent.

POC-PCR – what would be the recommendations and when should then test be performed?

The primary outcome of this study yielded a negative result. Adding POC-PCR to standard treatment did not result in a narrower antibiotic therapy when compared with SCO. However, it is possible that the results may have been different if the outcome or study design had been rethought. The most essential aim related to rational antibiotic treatment is not a narrow antibiotic treatment, as this is not always the goal, but a targeted treatment. Results indicated interesting findings concerning targeted treatment – patients might recover faster, delivering both clinical and economic results. The performance of TS would be the most appropriate before analysis by POC-PCR to ensure some level of quality criteria. However, the time to perform PCR may need to be reconsidered depending on the purpose of the POC-PCR – whether it is used for diagnostic or treatment purposes. If the goal is a targeted treatment, patients whom the ED physician is quite sure to have CAP would benefit from the POC-PCR immediately after their clinical assessment, as was performed in this study. However, POC-PCR could be beneficial after the performance of diagnostics tests such as blood tests, CXR or others where the diagnosis would be more delimited, contributing to initial targeted treatment. The test would be used more critically and benefit more patients. Nonetheless, even though the POC-PCR included testing for several bacteria and viruses, the panel does not include other agents, such as yeast, that are very rare but can also be causative of CAP. Therefore, POC-PCR should be added to the diagnostic process but should not replace culture for particular cases.

7. CONCLUSION

From a clinical and microbiological perspective, this thesis reflects the challenges in diagnosing CAP and provides new insights into optimising the diagnostic process through a *rapid* and *precise* diagnosis of CAP.

It can be concluded that when using only clinical characteristics and blood tests from the clinical assessment upon admission, a diagnostic prediction model is of limited value. This is because it cannot outperform the initial diagnosis made by ED physicians in the setting of this study. Therefore, adding new diagnostic tools to the diagnostic prediction model will be essential in future models before external validation. This study found that patients with LRTI had more samples of good quality collected from TS than FETIS, which is a prerequisite for a more *precise* diagnosis of LRTI. There was no difference in 'no or narrow' antibiotic prescriptions when POC-PCR was added to standard care, but POC-PCR is a *rapid* tool that might contribute to *earlier* and more targeted treatment and reducing the length of hospital stay. These three studies contribute knowledge and information to the diagnostic process and, when adapted to future research and implementation strategies, will assist in improving the diagnosis of CAP.

8. PERSPECTIVES

The next step will be to integrate the results of the three studies presented in this thesis with other results from the INDEED project to improve infectious disease diagnosis at the ED as the central focus in the diagnostic process illustrated in Figure 11 (155). The integration of the relevant findings from clinical characteristics; POC-PCR; POC urine flow cytometry; systemic inflammation biomarkers such as procalcitonin, suPAR, IL6, YKL40, Neutrophil gelatinase-associated lipocalin (NGAL); lung injury markers such as KL6, Surfactant-D; and imaging using ULDCT and focused lung ultrasound (FLUS) together with routine tests will provide an algorithm securing a rapid and precise diagnosis of the most common infections in EDs, including CAP. This will contribute to reducing diagnostic errors as well as the use of broad-spectrum antibiotics, resulting in positive patient and system outcomes, and thereby reducing the development of multi-resistant bacteria (32). Results from the algorithm could be automatically generated as part of the hospital IT system. However, multifaceted work is essential with the involvement of the diagnostic team and evaluation of the factors that could influence the diagnostic process. Therefore, a PPI is highly relevant before applying an algorithm or implementing a tool or new technologies.

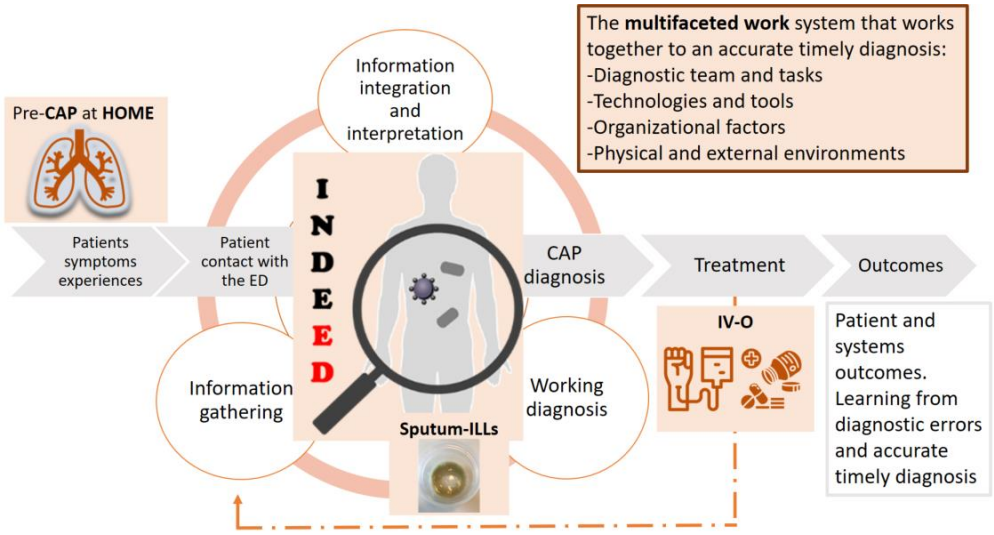


Figure 11: The INDEED project embedded in the diagnostic process
Pre-CAP at Home: visualise an earlier intervention for CAP patients. IV-PO: signalise an antibiotic intervention after an initial CAP diagnosis. IV: intravenous, O: oral.
Source: Diagnostic process modified from 'Improving Diagnosis in Health Care' (32).

The vision of future project initiatives will be generated based on the research strategy from Hospital Sønderjylland that is determined in the regional strategy for health research, Region South

Denmark's cooperation agreement with University of Southern Denmark and Hospital Sønderjylland's ordered plan, as well as the United Nation's (UN) global goals of ensuring healthy lives and promoting well-being for all ages (207). The research strategy has a particular focus on the patient involving research for the patient, clinical work and the development of the health system (207). New project initiatives to support this strategy will include interventions in coherent and safe patient processes based on the UN's global goals (207). A concrete proposal for future projects is listed below.

To improve a coherent patient course and develop an effective health system, initiatives to move the diagnostic process to an earlier stage – such as the patient's home – could be beneficial (see pre-CAP at Home, Figure 11). The dataset in Study III shows that 25% of the patients received antibiotic therapy within one month of admission, giving rise to the question of how long did the patients feel sick before contacting the health care system? Could CAP be predicted before the patient contacts the ED or the GP through a monitoring of biomarkers, symptoms and physical activity level? Making such a change would enable earlier and perhaps narrower treatment and may lessen treatment duration, further preventing crowding in EDs.

LRT specimens are widely used in the investigation of microorganisms. However, sputum is composed of a mixture of proteins, epithelial glycoproteins, lipids and enzymes (IgA, lactoferrin, phospholipids and lysozymes), and it has been reported that, compared with healthy controls, the immune responses levels of innate-like lymphocytes (ILLs) of sputum increase in CAP patients, indicating pulmonary infection (208). Further studies are needed to assess these inflammatory biomarkers' role in diagnosing CAP (Sputum-ILLs, Figure 11). However, in the future, they could contribute to a modern way by having a two-step analysis. A POC-PCR and inflammation biomarkers could supplement each other, ensuring a more accurate diagnosis.

Other initiatives to add knowledge to the diagnostic process and evaluate patient and system outcomes could be the testing of route administration (IV-O, Figure 11). Could the patient benefit from oral antibiotics at admission

instead of intravenous treatment? It could reduce hospital LOS, save many nurse hours and patients could possibly receive narrower antibiotic treatment.

Antimicrobial stewardship is essential and a cornerstone for successfully targeted and rational use of antibiotics. Antimicrobial stewardship ought to be embedded in future projects to acquire a rapid and precise diagnosis of CAP, to improve patient outcomes, reduce healthcare costs and prevent adverse effects and antibiotic resistance.

Finally, besides research in Danish EDs, and to support the research strategy from Hospital of Sønderjylland (207), I dream this research could be expanded with international collaborators. This could deliver a substantial contribution both to patient outcomes and public health and to controlling and reducing antibiotic resistance.

9. REFERENCES

1. Osler W, McCrae T. The Principles and Practice of Medicine. [Internet]: 9th ed. New York: D. Appleton and Company 1921 [cited 2023 February 13]. Available from: https://archive.org/details/principlesandpr00mccrgoog/mode/2up?view=theater
2. Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. P&T. 2015;40(4):277-283. doi:
3. Rossolini GM, Arena F, Pecile P, Pollini S. Update on the antibiotic resistance crisis. Curr Opin Pharmacol. 2014;18:56-60. doi: 10.1016/j.coph.2014.09.006
4. Sengupta S, Chattopadhyay MK, Grossart HP. The multifaceted roles of antibiotics and antibiotic resistance in nature. Front Microbiol. 2013;4:47. doi: 10.3389/fmicb.2013.00047
5. Discoverer stresses penicillin war boon: Fleming says drug will cure infected wounds in month. New York Times [ProQuest Historical Newspapers: New York Times 1923-Current file]. 1944 June 12
6. Fleming A. Penicillin ’s finder assays its future: Sir Alexander Fleming says improved dosage method is needed to extend use. New York Times [ProQuest Historical Newspapers: New York Times 1923-Current file]. 1945 June 26.
7. Bell BG, Schellevis F, Stobberingh E, Goossens H, Pringle M. A systematic review and meta-analysis of the effects of antibiotic consumption on antibiotic resistance. BMC Infect Dis. 2014;14 (1):13. doi: 10.1186/1471-2334-14-13
8. Munita JM, Arias CA. Mechanisms of antibiotic resistance. Microbiol Spectr. 2016;4(2). doi: 10.1128/microbiolspec.VMBF-0016-2015
9. Poku E, Cooper K, Cantrell A, Harnan S, Sin MA, Zanuzdana A, et al. Systematic review of time lag between antibiotic use and rise of resistant pathogens among hospitalized adults in Europe. JAC Antimicrob Resist. 2023;5(1):dlad001. doi: 10.1093/jacamr/dlad001
10. Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet. 2022;399(10325):629-655. doi: 10.1016/s0140-6736(21)02724-0
11. Cassini A, Högberg LD, Plachouras D, Quattrocchi A, Hoxha A, Simonsen GS, et al. Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the

EU and the European Economic Area in 2015: a population-level modelling analysis. Lancet Infect Dis. 2019;19(1):56-66. doi: 10.1016/s1473-3099(18)30605-4
12. World Health Organization. Antibiotic Resistance [Internet]: 2020 [updated 2020 July 31]. Available from: http://www.who.int/mediacentre/factsheets/antibiotic-resistance/en/.
13. Metlay JP, Waterer GW, Long AC, Anzueto A, Brozek J, Crothers K, et al. Diagnosis and treatment of adults with community-acquired pneumonia. An official clinical practice guideline of the American Thoracic Society and Infectious Diseases Society of America. Am J Respir Crit Care Med. 2019;200(7):e45-e67. doi: 10.1164/rccm.201908-1581ST
14. Eccles S, Pincus C, Higgins B, Woodhead M. Diagnosis and management of community and hospital acquired pneumonia in adults: summary of NICE guidance. BMJ. 2014;349. doi: 10.1136/bmj.g6722
15. Flemming SR. Antibiotikavejledning for Region Syddanmark: The region of Southern Denmark 2021 [updated 2022 September 22. Available from: https://ekstern.infonet.regionsyddanmark.dk/Files/Dokument547684.htm.
16. Rådet for Anvendelse af Dyr Sygehusmedicin. Baggrundsnotat for hensigtsmæssig anvendelse af antibiotika ved nedre luftvejsinfektioner i almen praksis og på hospital [Internet]: 2016 [cited 2021 August 08]. Available from: http://www.regioner.dk/media/3996/bgn-antibiotika-nedre-luftvejsinf-vers-1-0-november-2016-267967.pdf.
17. Woodhead M, Blasi F, Ewig S, Garau J, Huchon G, Ieven M, et al. Guidelines for the management of adult lower respiratory tract infections-Full version. Clin Microbiol Infect. 2011;17 (6 Suppl):E1-E59. doi: 10.1111/j.1469-0691.2011.03672.x
18. Zay Ya K, Win PTN, Bielicki J, Lambiris M, Fink G. Association between antimicrobial stewardship programs and antibiotic use globally: A systematic review and meta-analysis. JAMA Netw Open. 2023;6(2):e2253806. doi: 10.1001/jamanetworkopen.2022.53806
19. Trinh TD, Klinker KP. Antimicrobial stewardship in the emergency department. Infect Dis Ther. 2015;4(1 Suppl):39-50. doi: 10.1007/s40121-015-0084-8

20. Royal College of Physicians. Pneumonia in Adults: Diagnosis and Management – NICE Guideline [Internet]: [cited 2014 December 1]. Available from: https://www.rcplondon.ac.uk/guidelines-policy/pneumonia-adults-diagnosis-and-management-nice-guideline.
21. Meehan TP, Fine MJ, Krumholz HM, Scinto JD, Galusha DH, Mockalis JT, et al. Quality of care, process, and outcomes in elderly patients with pneumonia. JAMA. 1997;278(23):2080-2084. doi: 10.1001/jama.1997.03550230056037
22. Mandell LA. Community-acquired pneumonia: an overview. Postgrad Med. 2015;127(6):607-615. doi: 10.1080/00325481.2015.1074030
23. Bourcier JE, Paquet J, Seinger M, Gallard E, Redonnet JP, Cheddadi F, et al. Performance comparison of lung ultrasound and chest x-ray for the diagnosis of pneumonia in the ED. Am J Emerg Med. 2014;32(2):115-118. doi: 10.1016/j.ajem.2013.10.003
24. Hayden GE, Wrenn KW. Chest radiograph vs. computed tomography scan in the evaluation for pneumonia. J Emerg Med. 2009;36(3):266-270. doi: 10.1016/j.jemermed.2007.11.042
25. Savvateeva EN, Rubina AY, Gryadunov DA. Biomarkers of Community-Acquired Pneumonia: A Key to Disease Diagnosis and Management. Biomed Res Int. 2019;2019:1701276. doi: 10.1155/2019/1701276
26. Chandra A, Nicks B, Maniago E, Nouh A, Limkakeng A. A multicenter analysis of the ED diagnosis of pneumonia. Am J Emerg Med. 2010;28(8):862-865. doi: 10.1016/j.ajem.2009.04.014
27. Braykov NP, Morgan DJ, Schweizer ML, Uslan DZ, Kelesidis T, Weisenberg SA, et al. Assessment of empirical antibiotic therapy optimisation in six hospitals: an observational cohort study. Lancet Infect Dis. 2014;14(12):1220-1227. doi: 10.1016/s1473-3099(14)70952-1
28. Pandolfo AM, Horne R, Jani Y, Reader TW, Bidad N, Brealey D, et al. Understanding decisions about antibiotic prescribing in ICU: an application of the Necessity Concerns Framework. BMJ Qual Saf. 2022;31:199-210 doi: 10.1136/bmjqs-2020-012479
29. Webb BJ, Sorensen J, Jephson A, Mecham I, Dean NC. Broad-spectrum antibiotic use and poor outcomes in community-onset pneumonia: a cohort study. Eur Respir J. 2019;54(1):1900057. doi: 10.1183/13993003.00057-2019
30. Pletz MW, Jensen AV, Bahrs C, Davenport C, Rupp J, Witzenrath M, et al. Unmet needs in pneumonia research: a comprehensive

approach by the CAPNETZ study group. Respir Res. 2022;23(1):239. doi: 10.1186/s12931-022-02117-3
31. Newman-Toker DE, Pronovost PJ. Diagnostic errors--the next frontier for patient safety. JAMA. 2009;301(10):1060-1062. doi: 10.1001/jama.2009.249
32. Balogh EP, Miller BT, Ball JR, Committee on Diagnostic Error in Health Care. Board on Health Care Services; Institute of Medicine; Improving Diagnosis in Health Care. Washington, DC: National Academies Press (US); 2015.
33. Newman-Toker DE, Peterson SM, Badihian S, Hassoon A, Nassery N, Parizadeh D, et al. Diagnostic Errors in the Emergency Department: A Systematic Review [Internet]: Rockville (MD): Agency for Healthcare Research and Quality (US);2022 Dec. (Comparative Effectiveness Review, No. 258.) [Available from: https://www.ncbi.nlm.nih.gov/books/NBK588118/
34. Mandell LA, Wunderink RG, Anzueto A, Bartlett JG, Campbell GD, Dean NC, et al. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis. 2007;44(2 Suppl):S27-S72. doi: 10.1086/511159
35. Welte T, Torres A, Nathwani D. Clinical and economic burden of community-acquired pneumonia among adults in Europe. Thorax. 2012;67(1):71-79. doi: 10.1136/thx.2009.129502
36. Niederman MS, Luna CM. Community-acquired pneumonia guidelines: a global perspective. Semin Respir Crit Care Med. 2012;33(03):298-310. doi: 10.1055/s-0032-1315642
37. GBD 2016 Lower Respiratory Infections Collaborators. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Infect Dis. 2018;18(11):1191-1210. doi: 10.1016/s1473-3099(18)30310-4
38. McLaughlin JM, Khan FL, Thoburn EA, Isturiz RE, Swerdlow DL. Rates of hospitalization for community-acquired pneumonia among US adults: A systematic review. Vaccine. 2020;38(4):741-751. doi: 10.1016/j.vaccine.2019.10.101
39. World Health Organization. The top-10-causes-of-death [Internet]: 2020 [cited 2021 September 22]. Available from: https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death.

40. Statistics Denmark. [Internet]: 2023 [cited 2023 June 7]. Available from: https://www.statbank.dk/DODB1.
41. Vila-Corcoles A, Ochoa-Gondar O, Rodriguez-Blanco T, Raga-Luria X, Gomez-Bertomeu F, EPIVAC Study Group. Epidemiology of community-acquired pneumonia in older adults: a population-based study. Respir Med. 2009;103(2):309-316. doi: 10.1016/j.rmed.2008.08.006
42. Johnstone J, Eurich DT, Majumdar SR, Jin Y, Marrie TJ. Long-term morbidity and mortality after hospitalization with community-acquired pneumonia: a population-based cohort study. Medicine (Baltimore). 2008;87(6):329-334. doi: 10.1097/MD.0b013e318190f444
43. Weir DL, Majumdar SR, McAlister FA, Marrie TJ, Eurich DT. The impact of multimorbidity on short-term events in patients with community-acquired pneumonia: prospective cohort study. Clin Microbiol Infect. 2015;21(3):264.e267-264.e213. doi: 10.1016/j.cmi.2014.11.002
44. Di Pasquale MF, Sotgiu G, Gramegna A, Radovanovic D, Terraneo S, Reyes LF, et al. Prevalence and etiology of community-acquired pneumonia in immunocompromised patients. Clin Infect Dis. 2019;68(9):1482-1493. doi: 10.1093/cid/ciy723
45. Torres A, Peetermans WE, Viegi G, Blasi F. Risk factors for community-acquired pneumonia in adults in Europe: a literature review. Thorax. 2013;68(11):1057-1065. doi: 10.1136/thoraxjnl-2013-204282
46. Hammond A, Halliday A, Thornton HV, Hay AD. Predisposing factors to acquisition of acute respiratory tract infections in the community: a systematic review and meta-analysis. BMC Infect Dis. 2021;21(1):1254. doi: 10.1186/s12879-021-06954-3
47. Almirall J, Serra-Prat M, Bolíbar I, Balasso V. Risk Factors for Community-Acquired Pneumonia in Adults: A Systematic Review of Observational Studies. Respiration. 2017;94(3):299-311. doi: 10.1159/000479089
48. Prince AS, Mizgerd JP, Wiener-Kronish J, Bhattacharya J. Cell signaling underlying the pathophysiology of pneumonia. Am J Physiol Lung Cell Mol Physiol. 2006;291(3):L297-L300. doi: 10.1152/ajplung.00138.2006
49. Wunderink RG, Waterer GW. Community-acquired pneumonia: pathophysiology and host factors with focus on possible new approaches to management of lower respiratory tract infections.

Infect Dis Clin North Am. 2004;18(4):743-759, vii. doi: 10.1016/j.idc.2004.07.004
50. Girod S, Zahm JM, Plotkowski C, Beck G, Puchelle E. Role of the physiochemical properties of mucus in the protection of the respiratory epithelium. Eur Respir J. 1992;5(4):477-487. doi: 10.1183/09031936.93.05040477
51. Bartlett JG. Diagnostic test for etiologic agents of community-acquired pneumonia. Infect Dis Clin North Am. 2004;18(4):809-827. doi: 10.1016/j.idc.2004.08.002
52. Shoar S, Musher DM. Etiology of community-acquired pneumonia in adults: a systematic review. Pneumonia (Nathan). 2020;12:11. doi: 10.1186/s41479-020-00074-3
53. Mackenzie G. The definition and classification of pneumonia. Pneumonia (Nathan). 2016;8:14. doi: 10.1186/s41479-016-0012-z
54. Marrie TJ. Community-acquired pneumonia in the elderly. Clin Infect Dis. 2000;31(4):1066-1078. doi: 10.1086/318124
55. Jain S, Self WH, Wunderink RG, Fakhran S, Balk R, Bramley AM, et al. Community-acquired pneumonia requiring hospitalization among U.S. adults. N Engl J Med. 2015;373(5):415-427. doi: 10.1056/NEJMoa1500245
56. Kalil AC, Metersky ML, Klompas M, Muscedere J, Sweeney DA, Palmer LB, et al. Management of adults with hospital-acquired and ventilator-associated pneumonia: 2016 Clinical Practice Guidelines by the Infectious Diseases Society of America and the American Thoracic Society. Clin Infect Dis. 2016;63(5):e61-e111. doi: 10.1093/cid/ciw353
57. Mandell LA, Niederman MS. Aspiration pneumonia. N Engl J Med. 2019;380(7):651-663. doi: 10.1056/NEJMra1714562
58. Chalmers JD, Rother C, Salih W, Ewig S. Healthcare-associated pneumonia does not accurately identify potentially resistant pathogens: a systematic review and meta-analysis. Clin Infect Dis. 2014;58(3):330-339. doi: 10.1093/cid/cit734
59. Musher DM, Thorner AR. Community-acquired pneumonia. N Engl J Med. 2014;371(17):1619-1628. doi: 10.1056/NEJMra1312885
60. Lim WS, Baudouin SV, George RC, Hill AT, Jamieson C, Le Jeune I, et al. BTS guidelines for the management of community acquired pneumonia in adults: update 2009. Thorax. 2009;64 (3 Suppl):iii1-iii55. doi: 10.1136/thx.2009.121434

61. Aliberti S, Dela Cruz CS, Amati F, Sotgiu G, Restrepo MI. Community-acquired pneumonia. Lancet. 2021;398(10303):906-919. doi: 10.1016/s0140-6736(21)00630-9
62. Ebell MH, Chupp H, Cai X, Bentivegna M, Kearney M. Accuracy of signs and symptoms for the diagnosis of community-acquired pneumonia: a meta-analysis. Acad Emerg Med. 2020;27(7):541-553. doi: 10.1111/acem.13965
63. Moore M, Stuart B, Little P, Smith S, Thompson MJ, Knox K, et al. Predictors of pneumonia in lower respiratory tract infections: 3C prospective cough complication cohort study. Eur Respir J. 2017;50(5). doi: 10.1183/13993003.00434-2017
64. Htun TP, Sun Y, Chua HL, Pang J. Clinical features for diagnosis of pneumonia among adults in primary care setting: A systematic and meta-review. Sci Rep. 2019;9(1):7600. doi: 10.1038/s41598-019-44145-y
65. Müller B, Harbarth S, Stolz D, Bingisser R, Mueller C, Leuppi J, et al. Diagnostic and prognostic accuracy of clinical and laboratory parameters in community-acquired pneumonia. BMC Infect Dis. 2007;7:10. doi: 10.1186/1471-2334-7-10
66. World Health Organization. Aging and Health [Internet]: 2022 October 1 [cited 2022 October 28]. Available from: https://www.who.int/news-room/fact-sheets/detail/ageing-and-health.
67. Ravioli S, Germann C, Gygli R, Exadaktylos AK, Lindner G. Age- and sex-related differences in community-acquired pneumonia at presentation to the emergency department: a retrospective cohort study. Eur J Emerg Med. 2022;29(5):366-372. doi: 10.1097/mej.0000000000000933
68. Cillóniz C, Dominedò C, Pericàs JM, Rodriguez-Hurtado D, Torres A. Community-acquired pneumonia in critically ill very old patients: a growing problem. Eur Respir Rev. 2020;29(155):190126. doi: 10.1183/16000617.0126-2019
69. Metlay JP, Schulz R, Li YH, Singer DE, Marrie TJ, Coley CM, et al. Influence of age on symptoms at presentation in patients with community-acquired pneumonia. Arch Intern Med. 1997;157(13):1453-1459. doi: doi:10.1001/archinte.1997.00440340089009
70. Riquelme OR, Riquelme OM, Rioseco ZML, Gómez MV, Cárdenas G, Torres C. Neumonía adquirida en la comunidad en el anciano hospitalizado: Aspectos clínicos y nutricionales. [Community-

acquired pneumonia in the elderly: clinical and nutritional aspects]. Rev Med Chil. 2008;136(5):587-593. doi: 10.4067/S0034-98872008000500006
71. Zhang ZX, Yong Y, Tan WC, Shen L, Ng HS, Fong KY. Prognostic factors for mortality due to pneumonia among adults from different age groups in Singapore and mortality predictions based on PSI and CURB-65. Singapore Med J. 2018;59(4):190-198. doi: 10.11622/smedj.2017079
72. Arnold FW, Summersgill JT, Ramirez JA. Role of Atypical Pathogens in the Etiology of Community-Acquired Pneumonia. Semin Respir Crit Care Med. 2016;37(6):819-828. doi: 10.1055/s-0036-1592121
73. Shenkin SD, Fox C, Godfrey M, Siddiqi N, Goodacre S, Young J, et al. Delirium detection in older acute medical inpatients: a multicentre prospective comparative diagnostic test accuracy study of the 4AT and the confusion assessment method. BMC Med. 2019;17(1):138. doi: 10.1186/s12916-019-1367-9
74. Riquelme R, Torres A, el-Ebiary M, Mensa J, Estruch R, Ruiz M, et al. Community-acquired pneumonia in the elderly. Clinical and nutritional aspects. Am J Respir Crit Care Med. 1997;156(6):1908-1914. doi: 10.1164/ajrccm.156.6.9702005
75. Akhtar A, Hassali MAA, Zainal H, Ali I, Iqbal MS, Khan AH. Respiratory-tract infections among geriatrics: prevalence and factors associated with the treatment outcomes. Ther Adv Respir Dis. 2021;15:1753466620971141. doi: 10.1177/1753466620971141
76. Metlay JP, Kapoor WN, Fine MJ. Does this patient have community-acquired pneumonia? Diagnosing pneumonia by history and physical examination. JAMA. 1997;278(17):1440-1445. doi: 10.1001/jama.1997.03550170070035
77. Gentilotti E, De Nardo P, Cremonini E, Górska A, Mazzaferri F, Canziani LM, et al. Diagnostic accuracy of point-of-care tests in acute community-acquired lower respiratory tract infections. A systematic review and meta-analysis. Clin Microbiol Infect. 2022;28(1):13-22. doi: 10.1016/j.cmi.2021.09.025
78. Huang Y, Liu A, Liang L, Jiang J, Luo H, Deng W, et al. Diagnostic value of blood parameters for community-acquired pneumonia. Int Immunopharmacol. 2018;64:10-15. doi: 10.1016/j.intimp.2018.08.022
79. Long B, Long D, Koyfman A. Emergency medicine evaluation of community-acquired pneumonia: history, examination, imaging and

laboratory assessment, and risk scores. J Emerg Med. 2017;53(5):642-652. doi: 10.1016/j.jemermed.2017.05.035
80. Ebell MH, Bentivegna M, Cai X, Hulme C, Kearney M. Accuracy of biomarkers for the diagnosis of adult community-acquired pneumonia: a meta-analysis. Acad Emerg Med. 2020;27(3):195-206. doi: 10.1111/acem.13889
81. van Vugt SF, Broekhuizen BD, Lammens C, Zuithoff NP, de Jong PA, Coenen S, et al. Use of serum C reactive protein and procalcitonin concentrations in addition to symptoms and signs to predict pneumonia in patients presenting to primary care with acute cough: diagnostic study. BMJ. 2013;346:f2450. doi: 10.1136/bmj.f2450
82. van der Meer V, Neven AK, van den Broek PJ, Assendelft WJ. Diagnostic value of C reactive protein in infections of the lower respiratory tract: systematic review. BMJ. 2005;331(7507):26. doi: 10.1136/bmj.38483.478183.EB
83. Prendki V, Malézieux-Picard A, Azurmendi L, Sanchez JC, Vuilleumier N, Carballo S, et al. Accuracy of C-reactive protein, procalcitonin, serum amyloid A and neopterin for low-dose CT-scan confirmed pneumonia in elderly patients: A prospective cohort study. PLoS One. 2020;15(9):e0239606. doi: 10.1371/journal.pone.0239606
84. Wussler D, Kozhuharov N, Tavares Oliveira M, Bossa A, Sabti Z, Nowak A, et al. Clinical utility of Procalcitonin in the diagnosis of pneumonia. Clin Chem. 2019;65(12):1532-1542. doi: 10.1373/clinchem.2019.306787
85. Kamat IS, Ramachandran V, Eswaran H, Guffey D, Musher DM. Procalcitonin to distinguish viral from bacterial pneumonia: a systematic review and meta-analysis. Clin Infect Dis. 2020;70(3):538-542. doi: 10.1093/cid/ciz545
86. Savvateeva EN, Rubina AY, Gryadunov DA. Biomarkers of community-acquired pneumonia: a key to disease diagnosis and management. Biomed Res Int 2019;2019(2019):1701276. doi: 10.1155/2019/1701276
87. Müller DC, Kauppi A, Edin A, Gylfe Å, Sjöstedt AB, Johansson A. Phospholipid levels in blood during community-acquired pneumonia. PLoS One. 2019;14(5):e0216379. doi: 10.1371/journal.pone.0216379
88. Claessens YE, Debray MP, Tubach F, Brun AL, Rammaert B, Hausfater P, et al. Early chest computed tomography scan to assist

diagnosis and guide treatment decision for suspected community-acquired pneumonia. Am J Respir Crit Care Med. 2015;192(8):974-982. doi: 10.1164/rccm.201501-0017OC
89. Ibrahim D, Bizri AR, El Amine MA, Halabi Z. Chest computed tomography and chest X-ray in the diagnosis of community-acquired pneumonia: a retrospective observational study. J Int Med Res. 2021;49(8):3000605211039791. doi: 10.1177/03000605211039791
90. Kazerooni EA. High-resolution CT of the lungs. AJR Am J Roentgenol. 2001;177(3):501-519. doi: 10.2214/ajr.177.3.1770501
91. Brenner DJ, Hall EJ. Computed tomography--an increasing source of radiation exposure. N Engl J Med. 2007;357(22):2277-2284. doi: 10.1056/NEJMra072149
92. Kroft LJM, van der Velden L, Girón IH, Roelofs JJH, de Roos A, Geleijns J. Added value of ultra-low-dose computed tomography, dose equivalent to chest X-ray radiography, for diagnosing chest pathology. J Thorac Imaging. 2019;34(3):179-186. doi: 10.1097/rti.0000000000000404
93. van den Berk IAH, Kanglie M, van Engelen TSR, Altenburg J, Annema JT, Beenen LFM, et al. Ultra-low-dose CT versus chest X-ray for patients suspected of pulmonary disease at the emergency department: a multicentre randomised clinical trial. Thorax. 2023;78(5):515-522. doi: 10.1136/thoraxjnl-2021-218337
94. Orso D, Guglielmo N, Copetti R. Lung ultrasound in diagnosing pneumonia in the emergency department: a systematic review and meta-analysis. Eur J Emerg Med. 2018;25(5):312-321. doi: 10.1097/mej.0000000000000517
95. Staub LJ, Mazzali Biscaro RR, Kaszubowski E, Maurici R. Lung ultrasound for the emergency diagnosis of pneumonia, acute heart failure, and exacerbations of chronic obstructive pulmonary disease/asthma in Adults: a systematic review and meta-analysis. J Emerg Med. 2019;56(1):53-69. doi: 10.1016/j.jemermed.2018.09.009
96. Shimada T, Noguchi Y, Jackson JL, Miyashita J, Hayashino Y, Kamiya T, et al. Systematic review and metaanalysis: urinary antigen tests for Legionellosis. Chest. 2009;136(6):1576-1585. doi: 10.1378/chest.08-2602
97. Hyams C, Williams OM, Williams P. Urinary antigen testing for pneumococcal pneumonia: is there evidence to make its use uncommon in clinical practice? ERJ Open Res. 2020;6(1):00223-02019. doi: 10.1183/23120541.00223-2019

98. Said MA, Johnson HL, Nonyane BA, Deloria-Knoll M, O'Brien KL, Andreo F, et al. Estimating the burden of pneumococcal pneumonia among adults: a systematic review and meta-analysis of diagnostic techniques. PLoS One. 2013;8(4):e60273. doi: 10.1371/journal.pone.0060273
99. Sinclair A, Xie X, Teltscher M, Dendukuri N. Systematic review and meta-analysis of a urine-based pneumococcal antigen test for diagnosis of community-acquired pneumonia caused by Streptococcus pneumoniae. J Clin Microbiol. 2013;51(7):2303-2310. doi: 10.1128/jcm.00137-13
100. Gadsby NJ, Musher DM. The microbial etiology of community-acquired pneumonia in adults: from classical bacteriology to host transcriptional signatures. Clin Microbiol Rev. 2022;35(4):e0001522. doi: 10.1128/cmr.00015-22
101. Afshar N, Tabas J, Afshar K, Silbergleit R. Blood cultures for community-acquired pneumonia: are they worthy of two quality measures? A systematic review. J Hosp Med. 2009;4(2):112-123. doi: 10.1002/jhm.382
102. Carugati M, Aliberti S, Reyes LF, Franco Sadud R, Irfan M, Prat C, et al. Microbiological testing of adults hospitalised with community-acquired pneumonia: an international study. ERJ Open Res. 2018;4(4):00096-02018. doi: 10.1183/23120541.00096-2018
103. Fink JB. Forced expiratory technique, directed cough, and autogenic drainage. Respir Care. 2007;52(9):1210-1223. doi: Not found
104. Ogawa H, Kitsios GD, Iwata M, Terasawa T. Sputum gram stain for bacterial pathogen diagnosis in community-acquired pneumonia: a systematic review and bayesian meta-analysis of diagnostic accuracy and yield. Clin Infect Dis. 2020;71(3):499-513. doi: 10.1093/cid/ciz876
105. Joyce SM. Sputum analysis and culture. Ann Emerg Med. 1986;15(3):325-328. doi: 10.1016/s0196-0644(86)80576-5
106. Strickland SL, Rubin BK, Haas CF, Volsko TA, Drescher GS, O'Malley CA. AARC clinical practice guideline: effectiveness of pharmacologic airway clearance therapies in hospitalized patients. Respir Care. 2015;60(7):1071-1077. doi: 10.4187/respcare.04165
107. Nagendra S, Bourbeau P, Brecher S, Dunne M, LaRocco M, Doern G. Sampling variability in the microbiological evaluation of expectorated sputa and endotracheal aspirates. J Clin Microbiol. 2001;39(6):2344-2347. doi: 10.1128/jcm.39.6.2344-2347.2001

108. Weiszhar Z, Horvath I. Induced sputum analysis: step by step. Breathe. 2013;9(4):300-306. doi: 10.1183/20734735.042912
109. Paggiaro PL, Chanez P, Holz O, Ind P, Djukanović R, Maestrelli P, et al. Sputum induction. Eur Respir J 2002;37(Suppl):3s-8s. doi: 10.1183/09031936.02.00000302
110. Gunasekaran J, Saksena R, Jain M, Gaind R. Can sputum gram stain be used to predict lower respiratory tract infection and guide empiric antimicrobial treatment: Experience from a tertiary care hospital. J Microbiol Methods. 2019;166:105731. doi: 10.1016/j.mimet.2019.105731
111. Datta S, Shah L, Gilman RH, Evans CA. Comparison of sputum collection methods for tuberculosis diagnosis: a systematic review and pairwise and network meta-analysis. Lancet Glob Health. 2017;5(8):e760-e771. doi: 10.1016/s2214-109x(17)30201-2
112. Garcia-Vazquez E, Marcos MA, Mensa J, de Roux A, Puig J, Font C, et al. Assessment of the usefulness of sputum culture for diagnosis of community-acquired pneumonia using the PORT predictive scoring system. Arch Intern Med. 2004;164(16):1807-1811. doi: 10.1001/archinte.164.16.1807
113. Signori LG, Ferreira MW, Vieira LC, Muller KR, Mattos WL. Sputum examination in the clinical management of community-acquired pneumonia. J Bras Pneumol. 2008;34(3):152-158. doi: 10.1590/s1806-37132008000300005
114. Laurenzi GA, Potter RT, Kass EH. Bacteriologic flora of the lower respiratory tract. N Engl J Med. 1961;265:1273-1278. doi: 10.1056/nejm196112282652601
115. Miller JM, Binnicker MJ, Campbell S, Carroll KC, Chapin KC, Gilligan PH, et al. A guide to utilization of the microbiology laboratory for diagnosis of infectious diseases: 2018 update by the Infectious Diseases Society of America and the American Society for Microbiology. Clin Infect Dis. 2018;67(6):813-816. doi: 10.1093/cid/ciy584
116. Murray PR, Washington JA. Microscopic and baceriologic analysis of expectorated sputum. Mayo Clin Proc. 1975;50(6):339-344. doi: Not found
117. Geckler RW, Gremillion DH, McAllister CK, Ellenbogen C. Microscopic and bacteriological comparison of paired sputa and transtracheal aspirates. J Clin Microbiol. 1977;6(4):396-399. doi: 10.1128/jcm.6.4.396-399.1977

118. Miyashita N, Shimizu H, Ouchi K, Kawasaki K, Kawai Y, Obase Y, et al. Assessment of the usefulness of sputum Gram stain and culture for diagnosis of community-acquired pneumonia requiring hospitalization. Med Sci Monit 2008;14(4):CR171-CR176. doi: Not found
119. Fukuyama H, Yamashiro S, Kinjo K, Tamaki H, Kishaba T. Validation of sputum Gram stain for treatment of community-acquired pneumonia and healthcare-associated pneumonia: a prospective observational study. BMC Infect Dis. 2014;14:534. doi: 10.1186/1471-2334-14-534
120. Lee DH, Kim S. Clinical analysis of sputum gram stains and cultures to improve the quality of sputum cultures. Lab Med Qual Assur. 2020;42(1):33-39. doi: 10.15263/jlmqa.2020.42.1.33
121. Musher DM, Jesudasen SS, Barwatt JW, Cohen DN, Moss BJ, Rodriguez-Barradas MC. Normal respiratory flora as a cause of community-acquired pneumonia. Open Forum Infect Dis. 2020;7(9):ofaa307. doi: 10.1093/ofid/ofaa307
122. Del Rio-Pertuz G, Gutiérrez JF, Triana AJ, Molinares JL, Robledo-Solano AB, Meza JL, et al. Usefulness of sputum gram stain for etiologic diagnosis in community-acquired pneumonia: a systematic review and meta-analysis. BMC Infect Dis. 2019;19(1):403. doi: 10.1186/s12879-019-4048-6
123. Beveridge TJ. Use of the gram stain in microbiology. Biotech Histochem. 2001;76(3):111-118. doi: 10.1080/bih.76.3.111.118
124. Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA. Manual of clinical microbiology. 9th ed. Washington (DC): ASM Press; 2007.
125. Angeletti S. Matrix assisted laser desorption time of flight mass spectrometry (MALDI-TOF MS) in clinical microbiology. J Microbiol Methods. 2017;138:20-29. doi: 10.1016/j.mimet.2016.09.003
126. Cartuliares MB, Sundal LM, Gustavsson S, Skjøt-Arkil H, Mogensen CB. Limited value of sputum culture to guide antibiotic treatment in a Danish emergency department. Dan Med J. 2020;67(11):A11190641. Published 11192020 Oct 11190626. doi: Not found
127. Musher DM, Montoya R, Wanahita A. Diagnostic value of microscopic examination of Gram-stained sputum and sputum cultures in patients with bacteremic pneumococcal pneumonia. Clin Infect Dis. 2004;39(2):165-169. doi: 10.1086/421497

128. Ewig S, Schlochtermeier M, Goke N, Niederman MS. Applying sputum as a diagnostic tool in pneumonia: limited yield, minimal impact on treatment decisions. Chest. 2002;121(5):1486-1492. doi: 10.1378/chest.121.5.1486
129. Cartuliares MB, Skjøt-Arkil H, Mogensen CB, Skovsted TA, Andersen SL, Pedersen AK, et al. Gram stain and culture of sputum samples detect only few pathogens in community-acquired lower respiratory tract infections: secondary analysis of a randomized controlled trial. Diagnostics (Basel). 2023;13(4). doi: 10.3390/diagnostics13040628
130. Musher DM, Roig IL, Cazares G, Stager CE, Logan N, Safar H. Can an etiologic agent be identified in adults who are hospitalized for community-acquired pneumonia: results of a one-year study. J Infect. 2013;67(1):11-18. doi: 10.1016/j.jinf.2013.03.003
131. Murphy CN, Fowler R, Balada-Llasat JM, Carroll A, Stone H, Akerele O, et al. Multicenter evaluation of the BioFire FilmArray Pneumonia/Pneumonia Plus Panel for detection and quantification of agents of lower respiratory tract infection. J Clin Microbiol. 2020;58(7):e00128-00120. doi: 10.1128/jcm.00128-20
132. Gadsby NJ, Russell CD, McHugh MP, Mark H, Conway Morris A, Laurenson IF, et al. Comprehensive molecular testing for respiratory pathogens in community-acquired pneumonia. Clin Infect Dis. 2016;62(7):817-823. doi: 10.1093/cid/civ1214
133. Webber DM, Wallace MA, Burnham CA, Anderson NW. Evaluation of the BioFire FilmArray Pneumonia Panel for detection of viral and bacterial pathogens in lower respiratory tract specimens in the setting of a tertiary are academic medical center. J Clin Microbiol. 2020;58(7):e00343-00320. doi: 10.1128/jcm.00343-20
134. Zhou F, Wang Y, Liu Y, Liu X, Gu L, Zhang X, et al. Disease severity and clinical outcomes of community-acquired pneumonia caused by non-influenza respiratory viruses in adults: a multicentre prospective registry study from the CAP-China Network. Eur Respir J. 2019;54(2):1802406 doi: 10.1183/13993003.02406-2018
135. Yang S, Li H, Tang Y, Yu F, Ma C, Zhang H, et al. Multiplex tests for respiratory tract infections: the direct utility of the FilmArray Respiratory Panel in Emergency Department. Can Respir J. 2020;2020:6014563. doi: 10.1155/2020/6014563
136. Buchan BW, Windham S, Balada-Llasat JM, Leber A, Harrington A, Relich R, et al. Practical comparison of the BioFire FilmArray Pneumonia Panel to routine diagnostic methods and potential impact

on antimicrobial stewardship in adult hospitalized patients with lower respiratory tract infections. J Clin Microbiol. 2020;58(7):e00135-00120. doi: 10.1128/jcm.00135-20
137. Farrohknia N, Castrén M, Ehrenberg A, Lind L, Oredsson S, Jonsson H, et al. Emergency department triage scales and their components: a systematic review of the scientific evidence. Scand J Trauma Resusc Emerg Med. 2011;19:42. doi: 10.1186/1757-7241-19-42
138. Plesner LL, Iversen AKS, Langkjær S, Nielsen TL, Østervig R, Warming PE, et al. The formation and design of the TRIAGE study-baseline data on 6005 consecutive patients admitted to hospital from the emergency department. Scand J Trauma Resusc Emerg Med. 2015;23:106. doi: 10.1186/s13049-015-0184-1
139. Lindberg SØ, Lerche la Cour J, Folkestad L, Hallas P, Brabrand M. The use of triage in Danish emergency departments. Dan Med Bull. 2011;58(10):A4301. doi: Not found
140. Nordberg M, Lethvall S, Castrén M. The validity of the triage system ADAPT. Scand J Trauma Resusc Emerg Med. 2010;18(1 Suppl):P36. doi: 10.1186/1757-7241-18-S1-P36
141. Danish Emergency Process Triage. vitalparametre og kontaktårsagskort [Internet]: Version 2.0 [cited 2023 April 20]. Available from: http://www.deptriage.dk/.
142. Chalmers JD, Singanayagam A, Akram AR, Mandal P, Short PM, Choudhury G, et al. Severity assessment tools for predicting mortality in hospitalised patients with community-acquired pneumonia. Systematic review and meta-analysis. Thorax. 2010;65(10):878-883. doi: 10.1136/thx.2009.133280
143. Lim WS, van der Eerden MM, Laing R, Boersma WG, Karalus N, Town GI, et al. Defining community acquired pneumonia severity on presentation to hospital: an international derivation and validation study. Thorax. 2003;58(5):377-382. doi: 10.1136/thorax.58.5.377
144. Torres A, Cilloniz C, Niederman MS, Menéndez R, Chalmers JD, Wunderink RG, et al. Pneumonia. Nat Rev Dis Primers. 2021;7(1):25. doi: 10.1038/s41572-021-00259-0
145. Riedel S, Beekmann SE, Heilmann KP, Richter SS, Garcia-de-Lomas J, Ferech M, et al. Antimicrobial use in Europe and antimicrobial resistance in Streptococcus pneumoniae. Eur J Clin Microbiol Infect Dis. 2007;26(7):485-490. doi: 10.1007/s10096-007-0321-5
146. Nørskov-Lauritsen N, Pedersen N, Lam JUH, Nielsen HL, Kobel CM, Hansen DS. Haemophilus influenzae one day in Denmark:

prevalence, circulating clones, and dismal resistance to aminopenicillins. Eur J Clin Microbiol Infect Dis. 2021;40(10):2077-2085. doi: 10.1007/s10096-021-04247-w
147. Statens Serum Institut National & Food Institute, Technical University of Denmark. DANMAP 2021 Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. DK-2300 Copenhagen; 2021.
148. Jefferson T, Demicheli V, Rivetti D, Jones M, Di Pietrantonj C, Rivetti A. Antivirals for influenza in healthy adults: systematic review. Lancet. 2006;367(9507):303-313. doi: 10.1016/s0140-6736(06)67970-1
149. Lehnert R, Pletz M, Reuss A, Schaberg T. Antiviral medications in seasonal and pandemic influenza. Dtsch Arztebl Int. 2016;113(47):799-807. doi: 10.3238/arztebl.2016.0799
150. European Centre for Disease Prevention and Control. Prevention and Control of Seasonal Influenza [Internet]: 2023 [cited 2023 june 13]. Available from: https://www.ecdc.europa.eu/en/seasonal-influenza/prevention-and-control.
151. Saleem N, Kulkarni A, Snow TAC, Ambler G, Singer M, Arulkumaran N. Effect of corticosteroids on mortality and clinical cure in community-acquired pneumonia: a systematic review, meta-analysis, and meta-regression of randomized control trials. Chest. 2023;163(3):484-497. doi: 10.1016/j.chest.2022.08.2229
152. Dequin PF, Meziani F, Quenot JP, Kamel T, Ricard JD, Badie J, et al. Hydrocortisone in severe community-acquired pneumonia. N Engl J Med. 2023;388(21):1931-1941. doi: 10.1056/NEJMoa2215145
153. Moons KG, Altman DG, Reitsma JB, Ioannidis JP, Macaskill P, Steyerberg EW, et al. Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): explanation and elaboration. Ann Intern Med. 2015;162(1):W1-W73. doi: 10.7326/m14-0698
154. Moher D, Hopewell S, Schulz KF, Montori V, Gøtzsche PC, Devereaux PJ, et al. CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. BMJ. 2010;340:c869. doi: 10.1136/bmj.c869
155. Skjøt-Arkil H, Heltborg A, Lorentzen MH, Cartuliares MB, Hertz MA, Graumann O, et al. Improved diagnostics of infectious diseases in emergency departments: a protocol of a multifaceted multicentre

diagnostic study. BMJ Open. 2021;11(9):e049606. doi: 10.1136/bmjopen-2021-049606
156. Cartuliares MB, Skjøt-Arkil H, Rosenvinge FS, Mogensen CB, Skovsted TA, Pedersen AK. Effectiveness of expiratory technique and induced sputum in obtaining good quality sputum from patients acutely hospitalized with suspected lower respiratory tract infection: a statistical analysis plan for a randomized controlled trial. Trials. 2021;22(1):675. doi: 10.1186/s13063-021-05639-1
157. Lund H, Juhl CB, Andreasen J, Møller AM. Håndbog i litteratursøgning og kritisk læsning: redskaber til evidensbaseret praksis: Kbh.: Munksgaard; 2014.
158. Harris PA, Taylor R, Minor BL, Elliott V, Fernandez M, O'Neal L, et al. The REDCap consortium: building an international community of software platform partners. J Biomed Inform. 2019;95:103208. doi: 10.1016/j.jbi.2019.103208
159. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform. 2009;42(2):377-381. doi: 10.1016/j.jbi.2008.08.010H
160. BioFire FilmArray Pneumonia panel instruction booklet RFIT-ASY0144/145. Salt Lake City (UT); 2018.
161. Spieth PM, Kubasch AS, Penzlin AI, Illigens BM, Barlinn K, Siepmann T. Randomized controlled trials - a matter of design. Neuropsychiatr Dis Treat. 2016;12:1341-1349. doi: 10.2147/ndt.S101938
162. Cartuliares MB, Rosenvinge FS, Mogensen CB, Skovsted TA, Andersen SL, Pedersen AK, et al. Expiratory technique versus tracheal suction to obtain good-quality sputum from patients with suspected lower respiratory tract infection: a randomized controlled trial. Diagnostics (Basel). 2022;12(10):2504. doi: 10.3390/diagnostics12102504
163. Ding F, Han L, Yin D, Zhou Y, Ji Y, Zhang P, et al. Development and validation of a simple tool composed of items on dyspnea, respiration rates, and C-reactive protein for pneumonia prediction among acute febrile respiratory illness patients in primary care settings. BMC Med. 2022;20(1):360. doi: 10.1186/s12916-022-02552-5
164. Kitazawa T, Yoshihara H, Seo K, Yoshino Y, Ota Y. Characteristics of pneumonia with negative chest radiography in cases confirmed by

computed tomography. J Community Hosp Intern Med Perspect. 2020;10(1):19-24. doi: 10.1080/20009666.2020.1711639
165. Ray P, Birolleau S, Lefort Y, Becquemin MH, Beigelman C, Isnard R, et al. Acute respiratory failure in the elderly: etiology, emergency diagnosis and prognosis. Crit Care. 2006;10(3):R82. doi: 10.1186/cc4926
166. Dale AP, Marchello C, Ebell MH. Clinical gestalt to diagnose pneumonia, sinusitis, and pharyngitis: a meta-analysis. Br J Gen Pract. 2019;69(684):e444-e453. doi: 10.3399/bjgp19X704297
167. Heckerling PS, Gerber BS, Tape TG, Wigton RS. Prediction of community-acquired pneumonia using artificial neural networks. Med Decis Making. 2003;23(2):112-121. doi: 10.1177/0272989x03251247
168. Madison JM, Irwin RS. Expectorated sputum for community-acquired pneumonia: a sacred cow. Arch Intern Med. 2004;164(16):1725-1727. doi: 10.1001/archinte.164.16.1725
169. Pieterse A, Hanekom SD. Criteria for enhancing mucus transport: a systematic scoping review. Multidiscip Respir Med. 2018;13:22. doi: 10.1186/s40248-018-0127-6
170. Andrews J, Sathe NA, Krishnaswami S, McPheeters ML. Nonpharmacologic airway clearance techniques in hospitalized patients: a systematic review. Respir Care. 2013;58(12):2160-2186. doi: 10.4187/respcare.02704
171. Lewis LK, Williams MT, Olds TS. The active cycle of breathing technique: a systematic review and meta-analysis. Respir Med. 2012;106(2):155-172. doi: 10.1016/j.rmed.2011.10.014
172. Gursli S, Quittner A, Jahnsen RB, Skrede B, Stuge B, Bakkeheim E. Airway clearance physiotherapy and health-related quality of life in cystic fibrosis. PLoS One. 2022;17(10):e0276310. doi: 10.1371/journal.pone.0276310
173. Lidman C, Burman LG, Lagergren A, Ortqvist A. Limited value of routine microbiological diagnostics in patients hospitalized for community-acquired pneumonia. Scand J Infect Dis. 2002;34(12):873-879. doi: 10.1080/003655402100026967
174. Shariatzadeh MR, Marrie TJ. Does sputum culture affect the management and/or outcome of community-acquired pneumonia? East Mediterr Health J. 2009;15(4):792-799. doi: Not found
175. Pizzichini E, Pizzichini MM, Leigh R, Djukanović R, Sterk P. Safety of sputum induction. Eur Respir J. 2002;37(Suppl):9s-18s. doi: 10.1183/09031936.02.00000902

176. Langridge PJ, Sheehan RL, Denning DW. Microbial yield from physiotherapy assisted sputum production in respiratory outpatients. BMC Pulm Med. 2016;16:23. doi: 10.1186/s12890-016-0188-2
177. Fireman E. Induced sputum as a diagnostic tactic in pulmonary diseases. Isr Med Assoc J. 2003;5(7):524-527. doi: Not found
178. da Silva RM, Teixeira PJ, Moreira Jda S. The clinical utility of induced sputum for the diagnosis of bacterial community-acquired pneumonia in HIV-infected patients: a prospective cross-sectional study. Braz J Infect Dis. 2006;10(2):89-93. doi: 10.1590/s1413-86702006000200004
179. Morse M, Kessler J, Albrecht S, Kim R, Thakur R, Nthobatsang R, et al. Induced sputum improves the diagnosis of pulmonary tuberculosis in hospitalized patients in Gaborone, Botswana. Int J Tuberc Lung Dis. 2008;12(11):1279-1285. doi: Not found
180. Statens Serum Institut. Influenza Season 2020-2021 - Report on Disease Occurrence [Internet]: 2021 [updated 2021 July 6; cited 2023 February 1]. Available from: https://en.ssi.dk/surveillance-and-preparedness/surveillance-in-denmark/annual-reports-on-disease-incidence/influenza-season-2020-2021---report-on-disease-occurrence.
181. von Baum H, Welte T, Marre R, Suttorp N, Ewig S, CAPNETZ study group. Community-acquired pneumonia through Enterobacteriaceae and Pseudomonas aeruginosa: Diagnosis, incidence and predictors. Eur Respir J. 2010;35(3):598-605. doi: 10.1183/09031936.00091809
182. Murphy TF, Parameswaran GI. Moraxella catarrhalis, a human respiratory tract pathogen. Clin Infect Dis. 2009;49(1):124-131. doi: 10.1086/599375
183. Kommunernes Landsforening. Udvikling i indlæggelsestid på somatiske hospitaler 2009-2018. [Internet]: 2020 [cited 2023 May 5]. Available from: https://www.kl.dk/nyheder/momentum/2020/2020-16/de-aeldste-patienter-er-indlagt-i-markant-kortere-tid/.
184. Rutjes AW, Reitsma JB, Di Nisio M, Smidt N, van Rijn JC, Bossuyt PM. Evidence of bias and variation in diagnostic accuracy studies. CMAJ. 2006;174(4):469-476. doi: 10.1503/cmaj.050090
185. Huijskens EG, Rossen JW, Kluytmans JA, van der Zanden AG, Koopmans M. Evaluation of yield of currently available diagnostics by sample type to optimize detection of respiratory pathogens in

patients with a community-acquired pneumonia. Influenza Other Respir Viruses. 2014;8(2):243-249. doi: 10.1111/irv.12153
186. Prendki V, Huttner B, Marti C, Mamin A, Fubini PE, Meynet MP, et al. Accuracy of comprehensive PCR analysis of nasopharyngeal and oropharyngeal swabs for CT-scan-confirmed pneumonia in elderly patients: a prospective cohort study. Clin Microbiol Infect. 2019;25(9):1114-1119. doi: 10.1016/j.cmi.2018.12.037
187. Demars Y, Brahier T, Rotzinger DC, Brouillet R, Jaton K, Opota O, et al. Utility of polymerase chain reaction in nasopharyngeal swabs for identifying respiratory bacteria causing community-acquired pneumonia. Microbiol Spectr. 2022;10(3):e0037922. doi: 10.1128/spectrum.00379-22
188. Rodriguez RM, Fancher ML, Phelps M, Hawkins K, Johnson J, Stacks K, et al. An emergency department-based randomized trial of nonbronchoscopic bronchoalveolar lavage for early pathogen identification in severe community-acquired pneumonia. Ann Emerg Med. 2001;38(4):357-363. doi: 10.1067/mem.2001.118014
189. Meyer P, Rousseau H, Maillet JM, Thierry S, Sy O, Vicaut E, et al. Evaluation of blind nasotracheal suctioning and non-bronchoscopic mini-bronchoalveolar lavage in critically ill patients with infectious pneumonia: a preliminary study. Respir Care. 2014;59(3):345-352. doi: 10.4187/respcare.02356
190. Murray MP, Pentland JL, Turnbull K, MacQuarrie S, Hill AT. Sputum colour: a useful clinical tool in non-cystic fibrosis bronchiectasis. Eur Respir J. 2009;34(2):361-364. doi: 10.1183/09031936.00163208
191. Blackwelder WC. “Proving the null hypothesis” in clinical trials. Control Clin Trials. 1982;3(4):345-353. doi: 10.1016/0197-2456(82)90024-1
192. European Medicines Agency. Guideline on Missing Data in Confirmatory Clinical Trials [Internet]: 2010 july 2 [cited 2023 July 31]. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-missing-data-confirmatory-clinical-trials_en.pdf.
193. Rubin DB. Inference and missing data. Biometrika. 1976;63(3):581-592. doi: 10.1093/biomet/63.3.581
194. Cook T, Zea R. Missing data and sensitivity analysis for binary data with implications for sample size and power of randomized clinical trials. Stat Med. 2020;39(2):192-204. doi: 10.1002/sim.8428

195. Hot A, Bossuyt PM, Gerke O, Wahl S, Vach W, Zapf A. Randomized test-treatment studies with an outlook on adaptive designs. BMC Med Res Methodol. 2021;21(1):110. doi: 10.1186/s12874-021-01293-y
196. Vach W, Reiser V, Kolankowska I, Weber S, Rücker G. Design and evaluation of diagnostic studies [Internet]: Bundensministerium fur Bildung und Forschung; 2017 [cited 2023 May 1]. Available from: www.wb.uni-freiburg.de/inhalte/pdfs/oh-projekt/mfbv/book-diagnosticstudies_mfbv_ruecker_2017.
197. Lijmer JG, Bossuyt PM. Various randomized designs can be used to evaluate medical tests. J Clin Epidemiol. 2009;62(4):364-373. doi: 10.1016/j.jclinepi.2008.06.017
198. Fineberg H. Evaluation of computed tomography: achievement and challenge. AJR Am J Roentgenol 1978;131(1):1-4. doi: 10.2214/ajr.131.1.1
199. Mann CJ. Observational research methods. Research design II: cohort, cross sectional, and case-control studies. Emerg Med J. 2003;20(1):54-60. doi: 10.1136/emj.20.1.54
200. Das MK. Multicenter studies: relevance, design and implementation. Indian Pediatr. 2022;59(7):571-579. doi: Not found
201. Vest-Hansen B, Riis AH, Sørensen HT, Christiansen CF. Out-of-hours and weekend admissions to Danish medical departments: admission rates and 30-day mortality for 20 common medical conditions. BMJ Open. 2015;5(3):e006731. doi: 10.1136/bmjopen-2014-006731
202. Gamble C, Dudley L, Allam A, Bell P, Goodare H, Hanley B, et al. Patient and public involvement in the early stages of clinical trial development: a systematic cohort investigation. BMJ Open. 2014;4(7):e005234. doi: 10.1136/bmjopen-2014-005234
203. Schouten JA, Hulscher ME, Natsch S, Kullberg BJ, van der Meer JW, Grol RP. Barriers to optimal antibiotic use for community-acquired pneumonia at hospitals: a qualitative study. Qual Saf Health Care. 2007;16(2):143-149. doi: 10.1136/qshc.2005.017327
204. Jüni P, Altman DG, Egger M. Systematic reviews in health care: Assessing the quality of controlled clinical trials. BMJ. 2001;323(7303):42-46. doi: 10.1136/bmj.323.7303.42
205. Järvinen TL, Sihvonen R, Bhandari M, Sprague S, Malmivaara A, Paavola M, et al. Blinded interpretation of study results can feasibly and effectively diminish interpretation bias. J Clin Epidemiol. 2014;67(7):769-772. doi: 10.1016/j.jclinepi.2013.11.011

206. Gøtzsche PC. Blinding during data analysis and writing of manuscripts. Control Clin Trials. 1996;17(4):285-293. doi: 10.1016/0197-2456(95)00263-4
207. Kommunikationsafdelingen SHS. Sygehus Sønderjyllands Forskningsstrategi [Internet]: 2020 [cited 2023 August 2]. Available from: https://sygehussonderjylland.dk/forskning/forskning-pa-sygehus-sonderjylland.
208. Hannaway RF, Wang X, Schneider M, Slow S, Cowan J, Brockway B, et al. Mucosal-associated invariant T cells and Vδ2(+) γδ T cells in community acquired pneumonia: association of abundance in sputum with clinical severity and outcome. Clin Exp Immunol. 2020;199(2):201-215. doi: 10.1111/cei.13377

10. Appendices

Appendix A: Targeted treatment guidelines of CAP of the Region of Southern Denmark

Agents	First choice	Penicillin allergy	Therapy duration (iv* and oral)	Remarks
Streptococcus pneumoniae (Sensitive to penicillin)	Benzylpenicillin 1.2g (2 mill.IE) × 4 iv. Or Phenoxymethylpenicillin 0.6g -0.8g (1-1.2 mill.IE) × 4 oral	Cefuroxime 1.5g × 3 iv. Or Roxithromycin 300mg × 1 oral	5-7 days	Part of the normal microbiota in upper respiratory tract. May be contamination with pharyngeal microbiota.
Haemophilus influenzae (Sensitive to ampicillin)	Ampicillin 2g x 4 iv. or Benzylpenicillin 1.2g (2 mill. IE) x 4 iv. or Piv-ampicillin 1g x 3 oral or Amoxicillin 1g x 3 oral	Cefuroxime 1.5g × 3 iv. or Doxycycline 100mg x 2 first 24 hours oral followed by 100mg x 1 oral	5-7 days	
Haemophilus influenzae (Resistant to ampicillin)	Tazobactam/ Piperacillin 4g/0.5g x 3 iv. or Amoxicillin/ Clavulanic acid' 500/125mg x 4 oral	Cefuroxime 1.5g x 3 iv. or Doxycycline 100mg x 2 first 24 hours oral followed by	5-7 days	

		100mg x 1 oral		
Hemolytic streptococcus	Benzylpenicillin 1.2g (2 mill. IE) x 4 iv. or Phenoxymethylpenicillin 0.6-0.8 g (1-1.2 mill. IE) x 4 oral	Cefuroxime 1.5g x 3 iv. or Roxithromycin 300mg × 1 oral	7-10 days	Part of the normal microbiota in upper respiratory tract. These pathogens relatively often represent contamination with pharyngeal microbiota. Infection caused by Hemolytic streptococcus or Staphylococcus aureus will usually results in severe pneumonia.
Staphylococcus aureus (Sensitive to penicillin)	Benzylpenicillin 1.2g (2 mill. IE) x 4 iv. or Phenoxymethylpenicillin 0.6g -0.8g (1-1.2 mill.IE) x 4 oral	Cefuroxime 1.5g x 3 iv. Or Clindamycin 300mg x 3 oral	7-10 days	
Staphylococcus aureus (Sensitive to Dicloxacillin)	Cloxacillin 1g x 4 iv. or *Dicloxacillin 1g x 4 oral	Cefuroxime 1.5g x 3 iv. or Clindamycin 300mg x 3 oral	7-10 days	
Moraxella catarrhalis	Tazobactam/ Piperacillin 4g/0.5g x 3 iv. or Amoxicillin/ Clavulanic acid' 500/125mg x 3 oral	Cefuroxime 1.5g x 3 iv. or Roxithromycin 300mg × 1 oral	5-7 days	
Legionella pneumophila CURB=0 in non-immunocompromised patient	Azithromycin 500mg x 1 oral		5 days	or: Ciprofloxacin 500mg x 2 oral for 7-10 days
Legionella pneumophila Moderate or severe pneumonia or in	Azithromycin 500mg x 1 iv./oral		7-10 days	or: Ciprofloxacin 600/750mg x 2 iv./oral for 14 days

immunocompromised patient			
Mycoplasma pneumoniae or *Chlamydia pneumoniae*	Azithromycin 500mg x 1 oral	3 days	or: Roxithromycin 300mg x 1 oral for 10 days
Chlamydia psittaci	Doxycycline 200 mg x 1 iv./oral	10-14 days	or: Azithromycin 500mg x 1 iv./oral for 5-10 days

* Dicloxacillin cannot be used to treat pneumonia caused by the most common agents, and since the finding of *S.aureus* is frequently indicative of colonization, the regimen should be used with caution

Appendix B: Literature search strategy for all studies

Study I

Medline (Ovid):

1. pneumonia, bacterial/ OR pneumonia, viral/

2. cap.mp.

3. (communit* adj3 pneumon*).mp

4. Community-Acquired Infections/ OR "community-acquired pneumonia".mp.

5. symptoms.mp.

6. prediction.mp.

7. "associat*".m_titl.

8. diagnose.mp. OR exp Diagnosis/

9. respiratory tract diseases/ OR "signs and symptoms"/ OR medically unexplained symptoms/

10. "vital parameters".mp.

11. 1 OR 2 OR 3 OR 4

12. 5 OR 6 OR 7 OR 8 OR 9 OR 10

13. 11AND 12

14. limit 13 to (humans AND "all adult (19 plus years)")

15. "emergency department".mp.

16. 13 AND 14 AND 15 **(532)**

Study II

Medline (Ovid):

1. Respiratory Tract Infections.mp. OR exp Respiratory Tract Infections/

2. acute respiratory infection*.mp.

3. lower respiratory infection*.mp.

4. lower respiratory tract infection*.mp.

5. exp Pneumonia/

6. Pneumonia/ OR pneumonia.mp.

7. (pneumon* OR bronchopneumon* OR pleuropneumon*).tw.

8. exp Suction/

9. Tracheal suction.mp.

10. Tracheal aspirate.mp.

11. exp Sputum/

12. "Induced sputum".mp.

13. Saline Solution, Hypertonic/ OR Administration, Inhalation/

14. "Saline inhalation".mp.

15. Isotonic Solutions/

16. Isotonic saline inhalation.mp.

17. (forced expiratory adj1 technique).mp.

18. Cough*/ OR Cough technique.mp. OR "Respiratory Therapy"/

19. huff*.mp.

20. Forced exhalation technique.mp.

21. Active cycle of breathing.mp.

22. Airway clearance.mp.

23. "sputum collection".mp.

24. "sputum acquisition".mp.

25. "sputum submission".mp.

26. Sputum/di, mi [Diagnosis, Microbiology]

27. Gram stain.mp.

28. 1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7

29. 8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR 16 OR 17 OR 18 OR 19 OR 20 OR 21 OR 22 OR 23 OR 24 OR 25

30. 26 OR 27

31. 28 AND 29 AND 30

32. limit 31 to humans **(7710)**

Cinahl (EBSCO):

S1. acute respiratory infection* OR lower respiratory infection* OR lower respiratory tract infection* OR respiratory N3 infection

S2. MH pneumonia

S3. MH Empyema

S4. MH Bronchitis

S5. MH "Respiratory Tract Infections"

S6. S1 OR S2 OR S3 OR S4 OR S5

S7. "tracheal suction"

S8. "tracheal aspirate"

S9. "induced sputum"

S10. "Saline Solution"

S11. "Saline inhalation"

S12. "Cough technique"

S13. "airway clearance"

S14. MH sputum

S15. S7 OR S8 OR S9 OR S10 OR S11 OR S12 OR S13 OR S14

S16. S6 AND S15 **(530)**

PEDro:

1. Respiratory physiotherapy AND difficulty with sputum clearance AND chest

2. 1 AND randomized controlled trial **(555)**

3. 1 AND systematic reviews **(177)**

Study III

Medline (Ovid):

1. Pneumonia, Pneumococcal/ OR pneumonia.mp. OR Pneumonia, Aspiration/ OR Pneumonia, Viral/ OR exp Pneumonia/ OR Pneumonia, Staphylococcal/ OR Pneumonia, Bacterial/ OR Chlamydial Pneumonia/ OR Pneumonia, Mycoplasma/

2. cap.mp.

3. (communit* adj3 pneumon*).mp.

4. Community-Acquired Infections/ OR "community-acquired pneumonia".mp.

5. pcr.mp. OR exp Polymerase Chain Reaction/

6. Nucleic acid amplification tests.mp. OR exp Nucleic Acid Amplification Techniques/

7. Nucleic Acid Amplification Techniques/ OR naat.mp.

8. "sputum culture".mp. OR exp Sputum/

9. Culture Media/ OR exp Culture/ or culture.mp.

10. tracheal secretion.mp.

11. antibiotic.mp. OR exp Anti-Bacterial Agents/

12. antimicrobial.mp.

13. "biofire pneumonia panel plus".mp. OR Multiplex Polymerase Chain Reaction/

14. 1 or 2 or 3 or 4

15. 5 or 6 or 7 or 13

16. 8 or 9 or 10

17. 11 or 12

18. 14 and 15 and 16 and 17 **(453)**

Appendix C: Written consent and information form

Written consent form

Informeret samtykke til at deltage i et sundhedsvidenskabeligt projekt

Forbedret diagnostik af akutte infektioner

- Infectious Diseases in Emergency Departments (INDEED study)

Erklæring fra forsøgspersonen:
Jeg har fået skriftlig og mundtlig information, og jeg ved nok om formål, metode, fordele og ulemper til at sige ja til at deltage.

Jeg ved, at det er frivilligt at deltage, og jeg altid kan trække mit samtykke tilbage uden at miste mine nuværende eller fremtidige rettigheder til behandling.

Jeg giver hermed samtykke til at deltage i projektet og har fået en kopi af dette samtykkeark samt en kopi af den skriftlige information om projektet til eget brug.

Forsøgspersonens navn:
_____

Forsøgspersonens Cpr-nummer:
_____

Dato: _____ Underskrift: _____

Hvis der kommer nye væsentlige helbredsoplysninger frem om dig i forskningsprojektet vil du blive informeret. Vil du **frabede** dig information om nye væsentlige helbredsoplysninger, som kommer frem i forskningsprojektet, bedes du markere her: _____ (sæt x)

Erklæring fra den, der afgiver information:

Jeg erklærer, at forsøgspersonen har modtaget mundtlig og skriftlig information om forsøget.

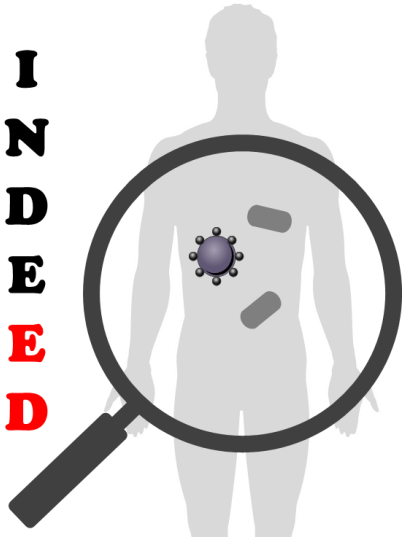
Efter min overbevisning er der givet tilstrækkelig information til, at der kan træffes beslutning om deltagelse i forsøget.

Navnet på den, der har afgivet information:

Dato: _____ Underskrift:_____

Deltagerinformation om deltagelse i videnskabeligt forskningsprojekt for personer, der indlægges akut med mistanke om lungebetændelse

Forbedret diagnostik af akutte infektioner



Infectious Diagnostics in Emergency Departments (INDEED study)

Et samarbejdsprojekt på tværs af specialer på Sygehus Sønderjylland, Sygehus Lillebælt og Odense Universitetshospital med udgangspunkt i Akutafdelingerne

Vi vil spørge, om du vil deltage i et videnskabeligt projekt?

Projektet handler om at blive bedre til at diagnosticere lungebetændelse på Akutafdelingen, så en målrettet behandling kan igangsættes så hurtigt som muligt.

Før du beslutter, om du vil deltage i projektet, skal du fuldt ud forstå, hvad projektet går ud på, og hvorfor vi gennemfører det. Vi vil derfor bede dig om at læse denne deltagerinformation grundigt.

Hvis du beslutter dig for at deltage, vil vi bede dig om at underskrive en samtykkeerklæring. Husk, at du har ret til at rådføre dig hos familie, venner eller bekendte. Du har også ret til betænkningstid før du underskriver, men da der er tale om en akut infektion, som kræver hurtig behandling, beder vi om, at du beslutter dig inden for 30 minutter.

Det er frivilligt at deltage i projektet. Du kan når som helst og uden at give en grund trække dit samtykke tilbage. Hvis du trækker dit samtykke om deltagelse i projektet tilbage, vil det ikke få konsekvenser for din videre behandling.

Projektets mål

De redskaber og undersøgelser, der eksisterer i dag til at diagnosticere lungebetændelse, har mange begrænsninger. Det udfordrer lægen i at stille en sikker diagnose inden for kort tid og igangsætte en målrettet behandling. Det kan få konsekvenser for den enkelte persons indlæggelsesforløb. Hvis man behandler med antibiotika som dækker flere bakterier end nødvendigt vil det også bidrage til udviklingen af bakterier, som er modstandsdygtige over for mange antibiotika.

Projektet har derfor til formål at finde bedre redskaber, som kan hjælpe lægen til at stille en sikker diagnose inden for få timer for personer, indlagt akut med mistanke om lungebetændelse.

Det undersøger projektet

Projektet vil undersøge

- hvilke symptomer, tegn og forhold, der kendetegner lungebetændelse og sygdomsgraden
- hvilke markører for infektion i blodet, der bedst kan identificere en lungebetændelse og sygdomsgraden
- om en ny metode til at måle bakterier i urinen er nyttig
- om en ny metode til at identificere bakterier i sekret fra lungerne er nyttigt
- om ultralydsundersøgelse og CT-skanning med meget lav strålingsrisiko kan bruges til at diagnosticere lungebetændelse

Plan for projektet

Projektet foregår på Fælles Akutmodtagelsen i Aabenraa, Sygehus Sønderjylland, på Akutafdelingen i Kolding, Sygehus Lillebælt, og på Fælle Akut Modtagelsen i Odense, Odense Universitetshospital. Fra februar 2021 til vinteren 2021/22 vil 500 voksne personer, som indlægges akut med mistanke om lungebetændelse på de tre akutafdelinger, blive inviteret til at deltage.

Personalet vil i forbindelse med din indlæggelse opsøge og informere dig om projektet, og tilbyde deltagelse i projektet. Da det er vigtigt at en akut infektion bliver behandlet hurtigt, vil vi bede dig om at tilkendegive din beslutning inden for en halv time.

Det indebærer deltagelse i projektet for dig

Deltagelse i projektet betyder, at du vil modtage den normale behandling, afdelingen tilbyder, og derudover få foretaget ekstra undersøgelser.

Vi vil stille dig nogle spørgsmål omkring dine symptomer, tidligere og aktuelle sygdomme, og hvordan du har det. Vi beder i den forbindelse adgang til din patientjournal, for at følge op på eventuelle tidligere indlæggelser, den aktuelle indlæggelse, og eventuelle indlæggelser inden for den næste måned efter du er udskrevet.

Vi vil tage 14mL ekstra blod svarende til 2 ekstra rør, når du alligevel får taget blodprøve, og hjælpe dig med at aflevere en urinprøve.

Af det sekret fra lungerne, som der bliver taget ifølge normal behandling, vil vi tage en lille del fra nogle af projektpersonerne, og undersøge det med en ny metode.

Det blod, urin og sekret fra lungerne, der indhentes til projektet, vil blive destrueret, når projektet er afsluttet.

Hvis du vælger at deltage, skal du have taget to ekstra skanninger af lungerne. 1) Ultralydsskanning som foretages på akutafdelingen og tager 5 min. 2) En CT-skanning som består af en skanning med meget lav strålingsrisiko, og en højopløselig CT-skanning, som er den mest præcise skanning, der benyttes på lungerne i dag. CT-skanningen vil i alt tage 10 min.

Dit samtykke vil give den forsøgsansvarlige, sponsor og dennes repræsentant direkte adgang til relevante helbredsoplysninger i journalen for at kunne gennemføre, overvåge og kontrollere projektet. I projektet vil behandlingen af personoplysninger følge databeskyttelsesloven og databeskyttelsesforordningen. Efter indsamling af de ønskede informationer vil dine persondata blive fjernet fra vores registreringssystem, og dit personnummer vil blive erstattet af en kode (pseudo-anonymisering).

Bivirkninger, risici, komplikationer og ulemper

Der er ingen eller kun få kendte risici eller bivirkninger ved at deltage i projektet. Alle prøvetagningsmetoder er velkendte og almindeligt anvendte procedurer, som vi har stor erfaring med og kendskab til. Der kan dog være risici ved undersøgelserne, som vi endnu ikke kender. Vi beder dig derfor om at fortælle, hvis du oplever problemer i forbindelse med prøvetagning og undersøgelser. Hvis vi opdager bivirkninger, som vi ikke allerede har fortalt dig om, vil du naturligvis blive orienteret med det samme, og du vil skulle tage stilling til, om du ønsker at fortsætte med prøvetagning og undersøgelser.

De ekstra blodprøver til projektet tages i forbindelse med de blodprøver, der alligevel tages ved indlæggelse. Risici og bivirkninger ved at få taget en blodprøver kan være ubehageligt, lette smerter og/eller blå mærker, og i nogle tilfælde besvimelse. I sjældne tilfælde kan der opstå en mindre blodansamling eller betændelse ved indstiksstedet.

Urinprøven kan som regel afleveres i et bæger, opsamlet ved toiletbesøg. Er du kateterbærer eller på grund af sygdom ikke selv kan lade vandet, kan det blive nødvendigt at hjælpe vandladningen på vej med et kateter, som er et tyndt plastikrør til indførsel i blæren via urinrøret. Denne procedure kan give let ubehag og eventuelt kortvarig mindre blødning fra slimhinderne.

Skanningerne er ikke forbundet med smerte, men du kan eventuelt opleve ubehag ved flytningen til CT-skanneren. Væsentligste risiko i forbindelse med deltagelse i projektet er den ekstra stråledosis som CT-skanningen medfører. Den ekstra stråledosis, du udsættes for, udgør i alt lidt mindre end den baggrundsstråling, som du normalt udsættes for i løbet af et år. Strålingen fra skanningen medfører en let øget risiko for udvikling kræft på ca. 0,01-0,1% og svarer til, at den samlede livstidsrisiko for kræft stiger fra 25% til 25,1%. Denne risiko vurderes dog betydningsløs i forhold til de risici, der i øvrigt er ved din aktuelle indlæggelse.

Dine prøvesvar

Ønsker du svar på de almindelige blod- og urinundersøgeler, kan du se det på www.sundhed.dk. Svar på de ekstra blod- og urinundersøgelser i projektet vil ikke fremkomme her, da vi ikke kender betydningen af resultaterne endnu. Har svarene et alarmerende resultat, vil den behandlende læge få besked og vil vurdere, om det har betydning for din behandling. Resultatet af den ekstra undersøgelse af sekret fra lungerne, som der vil kunne blive lavet i projektet, vil lægen, der behandler dig, blive orienteret om.

Skanningsresultaterne vil være synlige for lægerne, der behandler dig, og indgå i deres vurdering og behandling. Hvis vi skulle opdage noget, der kunne give os mistanke om andre sygdomme (f.eks. kræft), vil vi via din læge kontakte dig og tilbyde yderligere udredning. Bidrager skanningerne ikke med viden, der ændrer på din behandling eller diagnose, hører du ikke nærmere til skanningsresultatet.

Nytte ved projektet

Projektet er vigtigt for at vi fremadrettet kan gøre indlæggelsesforløbet for personer, der indlægges akut med mistanke om lungebetændelse, bedre. Projektet vil have en afgørende betydning for praksis på akutafdelingerne, og formentligt hvilken type antibiotika lægen ordinerer. Et mere målrettet antibiotikaforbrug vil bidrage til reduktion af bakterier, der er modstandsdygtige over for antibiotika, og dermed sikre at infektioner i fremtiden også kan behandles med antibiotika.

For dig personligt, vil deltagelse ikke umiddelbart have en betydning for dit behandlingsforløb. Hvis der skulle ske at være nogle særlige komplikationer til din aktuelle sygdom relateret til lungerne, vil vi dog med de ekstra scanninger formentlig hurtigere erkende dette.

Udelukkelse fra undersøgelse

Du vil udgå af dele af projektet, hvis nogle af undersøgelserne mislykkes af fx tekniske grunde eller hvis din behandlende læge vurderer, at det er for risikabelt for dig at deltage.

Adgang til projektets resultater

Projektets samlede resultater vil blive offentliggjort 2024 i videnskabelige tidsskrifter samt på sygehusenes hjemmesider. Resultater med relevans for beslutningstagere i sundhedsvæsenet vil blive offentliggjort i danske medier og tidsskrifter. Det sikres, at ingen deltagere kan genkendes i det, som offentliggøres. Har du interesse i at vide mere om projektets resultater, kan du efter offentliggørelse opsøge dem via http://www.sygehussonderjylland.dk/wm521282.

Vi håber, at du med denne information har fået tilstrækkeligt indblik i, hvad det vil sige at deltage i projektet, og at du føler dig rustet til at tage beslutningen om din eventuelle deltagelse. Hvis du vil vide mere, er du meget velkommen til at kontakte os. Information om dine rettigheder er vedlagt denne deltagerinformation sidst i dokumentet (Bilag 1).

Hvis du beslutter dig for at deltage i projektet, vil vi bede dig om at underskrive samtykkeerklæringen. Du kan vælge om du vil give samtykke til hele projektet eller kun dele af projektet. Det er frivilligt at deltage i projektet,

og du kan når som helst og uden at give en grund trække dit samtykke tilbage. Det vil ikke få konsekvenser for den videre behandling.

Yderligere oplysninger kan fås ved henvendelse til

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Tlf: 79971123

Initiativtagere til projektet

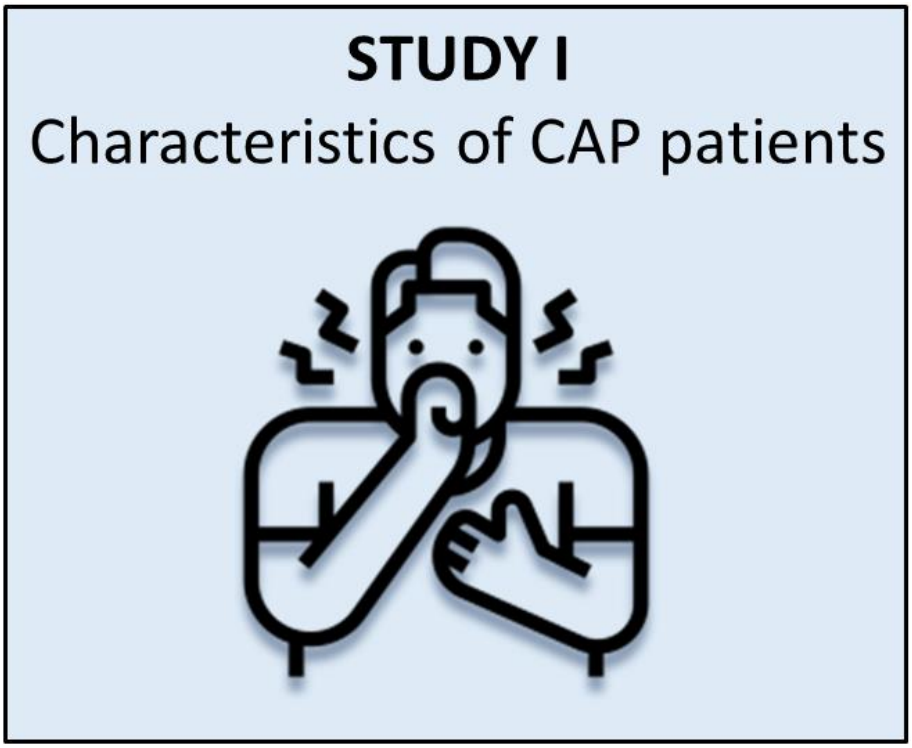
Projektet er primært udarbejdet i samarbejde mellem Akutafdeling, Biokemisk Afdeling og Mikrobiologisk Afdeling, og Radiologisk Afdeling på Sygehus Sønderjylland, Sygehus Lillebælt og Odense Universitets Hospital. Projektet er forankret på Sygehus Sønderjylland og Institut for Regional Sundhedsforskning på Syddansk Universitet, som er ansøgnings- og bevillingsansvarlige.

Økonomisk støtte til projektet

Projektet har fået økonomisk støttet i form af ph.d. stipendiater fra Syddansk Universitet (1.650.000kr), ph.d.-stipendiater fra Sygehus Sønderjylland (4.800.000kr) samt støtte til drift fra Region Syddanmark (500.000kr).
Forsøgsansvarlige har ingen økonomisk tilknytning til støttegivere eller andre interessenter i forsøget. Der vil ikke være en økonomisk kompensation til patienter, der deltager i projektet.

11. PAPERS

11.1 Paper I



Community-acquired pneumonia – Use of clinical characteristics of acutely admitted patients for the development of a diagnostic model: A cross-sectional multicentre study

Authors

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Strength and limitations

- This is a multicentre study with prospectively collected data
- Least absolute shrinkage and selection operator regression was used to establish a score for community-acquired pneumonia, and the performance of the diagnostic model was evaluated using the area under the receiver operating characteristic curve and calibration curves.
- This diagnostic prediction model could be improved in the future by adding diagnostic tools such as imaging or serological markers.

- External validation of the model using the clinical score for community-acquired pneumonia is lacking.

ABSTRACT

Objectives: This study aimed to describe the clinical characteristics of adults with acute community-acquired pneumonia (CAP) upon hospitalisation, evaluate their prediction performance for CAP and compare the performance of the model to the initial assessment of the physician.
Design: Cross-sectional, prospective, multicentre study.
Setting: The data originates from the INfectious DisEases in Emergency Departments study and were collected prospectively from patient interviews and medical records. The study included four Danish medical emergency departments (EDs) and was conducted between 1 March 2021 to 28 February 2022.
Participants: A total of 954 patients admitted with suspected infection were included in the study.

Primary and secondary outcome: The primary outcome was CAP diagnosis assessed by an expert panel.

Results: According to expert evaluation, CAP had a 28% prevalence. Thirteen diagnostic predictors were identified using Least absolute shrinkage and selection operator regression to build the prediction model: dyspnea, expectoration, cough, common cold, malaise, chest pain, respiratory rate (>20/min), oxygen saturation (< 96%), abnormal chest auscultation, leucocytes (<3,5 or >8,8 10E9/L) and neutrophilocytes (>7.5 10E9/L). In addition, C-reactive protein (<20 mg/L) and having no previous event of CAP

contributed negatively to the final model. The predictors yielded good prediction performance for CAP with an area under the ROC of 85% with a sensitivity of 86% (79%-93%) and specificity of 64% (57%-71%) using a 35% cut-off. However, the initial diagnosis made by the ED physician performed better, with 86% (84%-89%) sensitivity and 75% (72%-78%) specificity.

Conclusion: Typical respiratory symptoms combined with abnormal vital signs and elevated infection biomarkers were predictors for CAP upon admission to an ED. The clinical value of the prediction model is questionable in our setting. Further studies adding novel diagnostic tools and using imaging or serological markers are needed to improve the model, helping diagnose CAP in an ED setting more accurately.

Keywords: community-acquired pneumonia; diagnostic prediction model; emergency department

INTRODUCTION

Community-acquired pneumonia (CAP) is an increasing cause of hospitalisation and mortality, especially among elderly patients [1-5]. Early diagnosis and accurate treatment at the emergency department are essential to avoid serious complications such as bacteremia, sepsis, organ failure, and death [6] and to fight antimicrobial resistance [7].

Traditionally, the diagnosis of CAP generally requires a new infiltrate on chest x-ray with a clinically compatible syndrome [8]. These symptoms aren't sufficient to diagnose or exclude CAP, as they overlap with other diseases [8] and can be subtle in patients with advanced age and/or impaired immune systems [9, 10]. Chest x-ray is imprecise as diagnostic tool for CAP, risking under/over diagnosis [11, 12] and might not the optimal reference standard for CAP. This variability of clinical signs and symptoms combined with non-specific diagnostic tools [12], biomarkers [13, 14], and time-consuming microbiological tests [9] challenges physicians in differentiating CAP from other infections [10, 15].

The CAP population today has also changed with the increasing ageing [16], higher multimorbidities [17], and immunomodulatory treatments. Our knowledge of CAP symptoms and signs therefore need to be adapted to the actual population.

Previously, prediction models for the diagnosis of CAP have been developed based primarily on prognostic factors including severity assessment [18, 19], observations in a primary care setting only [20-22], or an outcome diagnosis based solely on the registered discharge diagnosis in the medical record or positive chest x-ray findings [22, 23]. A valid outcome diagnosis is essential.

An expert panel using several available information might be the best reference standard in pragmatic studies [11].

Therefore, there is a need to describe clinical characteristics of the current population of patients admitted with CAP and develop an improved diagnostic model to be used upon arrival at the emergency room that include physical examination, blood tests, vital signs, patient medical history, and healthcare expertise. Given the current diagnostic tool inaccuracies, an expert-panel-based diagnostic model is expected to surpass the ED physicians' initial accuracy.

Hypothesis and objectives

We hypothesised that developing of a diagnostic prediction model using well-defined clinical characteristics could assist an ED physician in an earlier, more precise CAP diagnosis. Therefore, the aim was to identify the clinical characteristics of adults admitted with CAP and evaluate their performance in a prediction model.

The objectives were:

- To investigate clinical characteristics in patients with a CAP diagnosis from i) all patients admitted with suspected infection and ii) patients suspected of CAP
- To develop and evaluate a diagnostic model to identify patients with CAP among ED patients suspected of infection and to compare the performance of the model to the initial assessment of the ED physician

METHODS

The study was reported following "The Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis" (TRIPOD) statement [24] and conducted in agreement with the Declaration of Helsinki-Ethical principle for medical research involving human subjects. The protocol was approved by the Regional Committee on Health Research Ethics for Southern Denmark (S- 20200188), registered by the Danish Data Protection Agency (no. 20/60508), and by ClinicalTrials.gov (NCT04681963).

Study design, source of data, and setting

This study had a prospective, analytical cross-sectional, multicentre design. The data originates from the INfectious DisEases in Emergency Departments (INDEED) study. The published study protocol provides further detailed information [25]. Four Danish medical EDs participated, covering around 775,000 inhabitants, during March 1, 2021, to February 28, 2022.

In Denmark, patients can be directed to various specialties within the ED, e.g. medical, gastrointestinal surgery, cardiology, orthopedics, gynecology, psychiatry, and intensive care [26]. Suspected infection cases usually are assigned to the medical ED.

Participants

Adult patients (≥ 18 years) admitted to the medical ED were eligible to participate. Patients were included if the ED physician suspected infection and if the patients could provide verbal and written consent. The exclusion criteria included: i) need for urgent, life-saving treatment, ii) transferal to intensive care, iii) admission within the last fortnight, iv) verified SARS-

CoV-2 infection at the time of admission or within 14 days before admission, v) severe immunodeficiencies (HIV positive, with a cluster of differentiation 4 cell count <200) or treatment with immunosuppressive medicine (Anatomical Therapeutic Chemical classification L04A), corticosteroids (>20 mg/day prednisone or equivalent for >14 days within the last 30 days) or chemotherapy within 30 days.

Recruitment and data collection

Six project assistants with a healthcare background (three physicians, one physiotherapist, and two final-year medical students) were responsible for inclusion and data collection from Mondays to Fridays, 8 am to 8 pm. A project assistant consecutively identified eligible patients from the patient management system. Immediately following the initial clinical assessment, the project assistant asked the ED physician whether an infection was suspected and the most likely infection focus (CAP, urinary tract infection, or unknown origin). Generally, the clinical assessment took place within 30 minutes upon admission before blood tests or imaging was ordered, and therefore, the ED physician often had only information on the patient's signs, symptoms, and vital parameters. The study assistant collected verbal and written consent from eligible patients. All data collected was registered in the electronic study database REDCap (Research Electronic Data Capture) [27].

Outcome

The outcome was the diagnosis of CAP. An expert panel was established consisting of pairs of experienced infectious diseases and emergency medicine specialists at each site. They conducted a patient file audit and determined the final diagnosis based on all clinical information registered

within the first week of ED admission. The information included routine laboratory tests of blood, -urine, and -sputum. In addition, polymerase chain reaction test of sputum, urine flow cytometry, chest x-ray, and chest computed tomography (CT) were available for some patients. The experts were blinded to each other and independently registered their assessments in a standardized electronic template [27] in the study database. Disagreements were discussed until a consensus was reached.

Predictors

All clinical characteristics were collected upon arrival at the ED. Symptoms, demographic data, and lifestyle factors were registered during a standardised bedside interview with the patient. In addition, information about vital parameters, comorbidities, medical treatment, and blood tests were collected from the patient's medical record. The project assistants collecting data were blinded to the final diagnosis.

Several candidate predictors (70) were selected from the literature and discussed with the specialists and project group [20, 28-37]. The pre-specified potential predictors with their measurement units, groups, cut-offs, and which considerations/assumptions of inclusion were selected and are described in Supplemental material, Supplementary Table S1.

- Demographic information, lifestyle factors, and comorbidities: age, sex, civil status, employment, nursing home residence, smoking, and alcohol consumption, body mass index (BMI), level of physical activity, activities of daily living score, dementia, respiratory, neurological, cardiovascular, endocrinological, nephrological and gastrointestinal comorbidities were collected.

-Patient symptoms the last two weeks before admission: malaise, fatigue, headache, dizziness, altered mental status, e.g. confusion, dyspnea, malnutrition, cough, secretions from the respiratory tract, sore throat, common cold, fever feeling, chest pain, peripheral oedema, nausea, vomiting, decreased appetite, abdominal pain, diarrhoea, and pain in muscles and joints including back pain were collected.

-Severity assessment, clinical parameters with cut-offs based on National Early Warning Score (NEWS) [38] used at the arrival of the ED and the use of medications: CURB-65 ≥3 (confusion, uremia, respiratory rate, blood pressure, age > 65 years), triage [39], Glasgow coma scale (GCS), oxygen saturation <96%, heart rate <51 or >90/min, blood pressure (systolic <111 or >219, diastolic ≤60 mmHg), respiratory rate >20/min, temperature > 38ºC, abnormal chest auscultation, abdominal tenderness, polypharmacy (≥ 5 medications), use of analgesics, and vaccination status (SARS-CoV-2, pneumococcus, influenza) were recorded.

-Blood tests with cut-offs routinely applied at our institutions: haematocrit (%), hemoglobin (mmol/L), leukocytes (10E9/L), platelets (10E9/L), neutrophils (10E9/L), lymphocytes (10E9/L), albumin g/L, creatinine (µmol/L), blood urea nitrogen (mmol/L), sodium (mmol/L), prothrombin, bilirubin (µmol), glucose (mmol/L), and CRP (mg/L) were recorded.

Statistical methods

The study sample size was estimated based on the University Hospital of Southern Denmark data. We estimated a need for at least 700 patients admitted with suspected infection. Of those, four hundred patients should be with suspected CAP and two hundred patients should have verified CAP for sufficient multivariable regression analysis. Descriptive statistics for baseline characteristics of the patients were conducted for the 70 potential predictors based on the data from the INDEED study [25]. Data were presented as means and standard deviations (SD), or medians and interquartile ranges (IQRs) for continuous variables, and numbers (n) and percentages (%) for categorical and binary variables. Extensive univariate logistic regression analyses were performed to examine the unadjusted association between each candidate predictor and the outcome CAP. Results of univariate analyses were reported with odds ratio (OR), 95% confidence intervals (CI), and statistical significance levels were two-sided reported with a p-value of <0.05 to present a descriptive overview of the individual's associations in the population. Complete case analyses were performed and the predictors were dichotomised or categorised and presented with percentages (%) for inclusion in the final model. The least absolute shrinkage and selection operator (LASSO) multivariable regression was performed with a random split-sample to develop and validate the model, using 20 % of the data for internal cross-validation. The model calibration was assessed using a likelihood ratio test, and recalibration was done based on the calibration belt and the optimal predicted proportion. In the model, age (≥75 years old) was considered as an effect modifier based on several studies showing differences in symptoms and signs for a CAP diagnosis in older adults [33, 40-42]. An exploratory approach was conducted for the clinical characteristics to achieve a model

with the best predictive performance, testing their performance as continuous, dichotomous, or categorical variables. In addition, the receiver-operator characteristic (ROC) curve was created to estimate the model's accuracy, and the area under the ROC curve (AUC) visualized the discrimination between true positives and negatives. The sensitivity, specificity, and positive and negative predictive values with 95% CI were calculated using the best threshold criteria of the predicted probability of the ROC curve.The same threshold was implemented in developing a CAP score, including the predictor variables. A CAP score> 0 represents the presence of CAP, and < 0 indicates the absence of CAP. Sensitivity, specificity, and positive and negative predictive values with 95% CI were calculated from the initial diagnosis made by the ED physician. Analyses were performed using STATA 17.0 (Texas, USA).

Patient and public involvement

Patients and/or the public were not directly involved in this study.

RESULTS

Participants

We recruited 954 patients admitted to the ED with suspected infection, representing 43% screened for eligibility. Of those, the attending physician suspected 402 (42%) had CAP. Patients with verified CAP diagnosis by the expert panel comprised of 265 (28%) of the recruited patients (Figure 1).

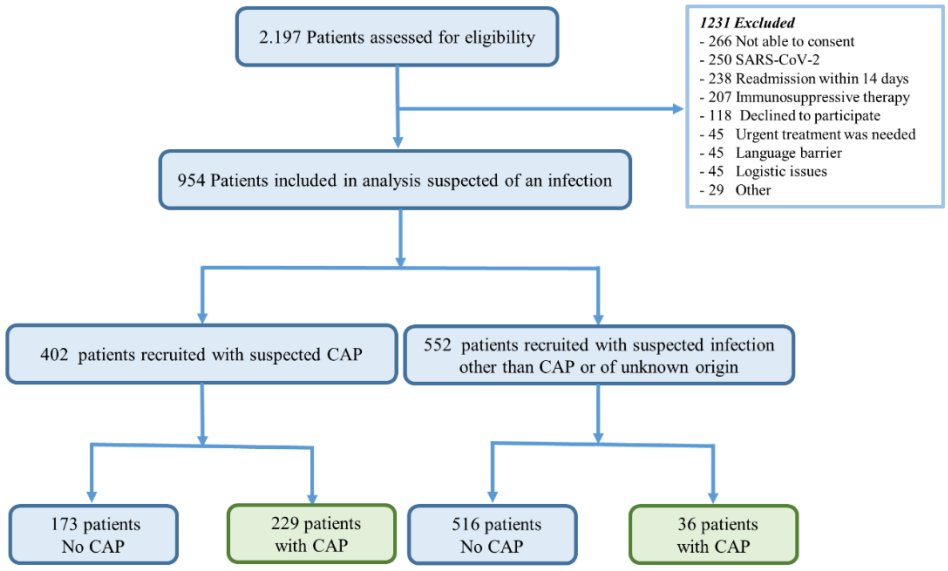

Figure 1: Trial population, green boxes showing the numbers of patients with CAP.

Characteristics of patients with suspected infections

We compared the clinical characteristics of patients with verified CAP to patients with suspected infection (954) without verified CAP. Median age for patients with verified CAP was 75 years (IQR: 63.5; 82.0), and over half admitted with suspected infection were males (53.8%). Univariate analysis revealed that verified CAP patients were more often previous smokers [OR 1.83 (CI: 1.30-2.57) p<0.001] with smoking history compared non-CAP cases. Strongly independent predictors for CAP were symptoms such as dyspnea, cough, expectoration, chest pain, and cold symptoms (all p<0.001). Compared to patients without CAP, the risk of having CAP increased fivefold if the patient had chest auscultation abnormalities [OR 5.67 (CI: 4.15-7.75) p<0.001] and decreased by half in case of abdominal tenderness by palpation [OR 0.52 (CI: 0.35-0.78) p=0.002]. CAP patients often had comorbidities

related to other pulmonary diseases (p<0.001) and had more previous CAP infections (p<0.001). These patients were more acutely ill when assessed by triage (p<0.001), with fever > 38ºC (p=0.036), higher respiratory rate [median 20.0 (IQR 18.0; 24.0) p<0.001], higher heart rate [mean 93.2 (SD 18.9) (p<0.001], and lower oxygen saturation [median 95.0 (IQR: 93.0; 97.0) p<0.001]. Patients with verified CAP had a median CRP of 125.0 (IQR: 57.0; 203.5) versus 82.0 (IQR: 19.0; 172.0) (p<0.001) compared to the rest of the population and higher levels of neutrophilocytes (p<0.001) and leucocytes (p<0.001). Furthermore, lymphocytes yielded a p-value of 0.018. Patients with verified CAP were more often vaccinated against SARS-CoV-2 (p=0.033) and influenza (p=0.025), but no differences were found regarding pneumococcal vaccination. Table 1 presents the characteristics of the population with statistically significant results of the unadjusted association between each predictor for patients with verified and not verified CAP. See Supplementary Table S2 for the 70 exploratory results from continuous, dichotomous, and categorical variables tested in the diagnostic prediction model.

Table 1: Characteristics of the population with suspected infection (n=954).

Characteristics	Patients suspected of infection at admission		Missings n (%)	OR (95% CI)	p-value
	CAP n (%)	Not CAP n (%)			
Total of patients	265 (27.8)	689 (72.2)	0 (0.0)	-	-
LIFESTYLE FACTORS					
Smoking status			33 (3.5)		
No	66 (26.0)	257 (38.5)		1 (reference)	
Current smoker	54 (21.3)	125 (18.7)		1.68 (1.10-2.55)	0.015
Previous smoker	134 (52.8)	285 (42.7)		1.83 (1.30-2.57)	<0.001
SYMPTOMS					
Malaise	173 (67.8)	386 (58.7)	41 (4.3)	1.48 (1.09-2.01)	0.010
Dyspnea	171 (67.3)	208 (31.5)	39 (4.1)	4.48 (3.29-6.11)	<0.001
Cough	173 (68.1)	185 (28.0)	39 (4.1)	5.49 (4.01-7.52)	<0.001
Expectoration	140 (55.1)	139 (21.0)	39 (4.1)	4.61 (3.38-6.28)	<0.001
Sore throat	39 (15.4)	65 (9.8)	39 (4.1)	1.66 (1.08-2.54)	0.019
Common cold	45 (17.7)	50 (7.6)	39 (4.1)	2.63 (1.70-4.05)	<0.001
Chest pain	71 (28.1)	97 (14.7)	40 (4.2)	2.26 (1.60-3.21)	<0.001
Oedema	10 (4.0)	69 (10.4)	40 (4.2)	0.35 (1,17-0.69)	0.002
Vomiting	40 (15.8)	150 (22.6)	38 (4.0)	0.64 (0.43-0.94)	0.023
Gastrointestinal pain	40 (15.8)	153 (23.1)	38 (4.0)	0.62 (0.42-0.91)	0.016
Muscular pain	79 (31.3)	265 (40.3)	44 (4.6)	0.67 (0.49-0.92)	0.013
COMORBIDITIES					
Pulmonary diseases	105 (39.6)	164 (23.8)	0 (0.0)	2.10 (1.55-2.84)	<0.001
Prior pneumonia			100(10.5)		
No	79 (33.3)	331 (53.6)		1 (reference)	
Yes, one time	50 (21.1)	130 (21.1)		1.61 (1.07-2.42)	0.022
Yes, more than one time	108 (45.6)	156 (25.3)		2.90 (2.05-4.10)	<0.001
VACCINATIONS					
SARS-CoV-2 †	222 (83.8)	534 (77.5)	0 (0.0)	1.49 (1.03-2.17)	0.033
Influenza	191 (72.1)	444 (64.4)	0 (0.0)	1.42 (1.04-1.94)	0.025
CLINICAL ASSESSMENT					
Abnormal chest auscultation*	168 (65.4)	161 (25.0)	52 (5.4)	5.67 (4.15-7.75)	<0.001

Abdominal tenderness	37 (15.0)	155 (25.0)	86 (9.0)	0.52 (0.35-0.78)	0.002
SEVERITY ASSESSMENT					
Triage**			59 (6.2)		
Green/Blue	37 (14.8)	146 (22.6)		1 (reference)	
Yellow	126 (50.4)	353 (54.7)		1.40 (0.93-2.13)	0.105
Red/Orange	87 (34.8)	146 (22.6)		2.35 (1.50-3.67)	<0.001
VITAL PARAMETERS					
Respiratory rate >20/min	124 (47.0)	161 (23.5)	5 (0.5)	2.88 (2.13-3.88)	<0.001
Oxygen saturation < 96 %	162 (61.1)	231 (33.7)	4 (0.4)	3.09 (2.30-4.14)	<0.001
Heart rate <51 or >90/min	148 (55.8)	312 (45.3)	1 (0.1)	1.52 (1.14-2.02)	0.003
Fever > 38ºC	77 (29.3)	156 (22.7)	5 (0.5)	1.40 (1.02-1.93)	0.036
BLOOD TESTS					
Leukocytes <3.5 or > 8.8 10E9/L	214 (80.8)	456 (66.2)	0 (0.0)	2.14 (1.52-3.02)	<0.001
Neutrophilocytes > 7.5 10E9/L	187 (71.1)	362 (53.2)	10 (1.0)	2.16 (1.59-2.94)	<0.001
Lymphocytes† <1.00 or > 4.00 10E9/L	53 (55.2)	92 (40.9)	633(66.3)	1.78 (1.10-2.88)	0.018
C-Reactive protein mg/L			0 (0.0)		
<20 mg/L	21 (7.9)	175 (25.4)		1 (reference)	
21-99 mg/L	86 (32.5)	205 (29.8)		3.49 (2.08-5.86)	<0.001
≥ 100 mg/L	158 (59.6)	309 (44.8)		4.26 (2.60-6.96)	<0.001

The predictors in the table are those dichotomised or categorised as they were later incorporated into the final diagnostic model. Only statistically significant results of the unadjusted association between each candidate predictor and the outcome CAP are presented. *Abnormal chest auscultation: Any abnormal findings such as crackles and rhonchi. ** Triage: Danish emergency process triage [39]. † Variables not included in the multivariate model.

Characteristics of patients suspected of CAP

Using the 70 candidate predictors, we compared clinical characteristics of patients with verified CAP to patients with suspected (402) but not verified CAP.

Statistically significant differences are shown in Table 2. Of the 402 patients with suspected CAP, half of the patients, 229 (57%) had verified CAP. Patients with suspected CAP had a median age of 74.0 (IQR: 62.0; 82.0), and half were male (52.7%). Patients with verified CAP reported more respiratory symptoms, such as cough (p=0.009) and expectoration (p=0.037), and more gastrointestinal symptoms, such as nausea (p=0.033) and loss of appetite (p=0.030), compared to those without CAP. Fewer patients with verified CAP had a CURB-65 ≥3 (p=0.047), and more patients had oxygen saturation <96% (p<0.001), a heart rate of <51 or >100bpm/min (p=0.045), and fever >38 ºC (p=0.011). Elevated infection biomarkers (leukocytes, neutrophilocytes, CRP, all p<0.001), and plasma natrium (p<0.001) were highly associated with CAP. Fewer patients with CAP had plasma bilirubin values of <5 or >25 mmol/L (p=0.045) (Table 2).

Table 2: Characteristics of the population with suspected CAP (n=402) by the physician at admission.

Characteristics	Patients suspected of CAP at admission		Missings n (%)	OR (95% CI)	p-value
	CAP n (%)	Not CAP n (%)			
Total of patients	229 (57.0)	173 (43.0)	0 (0.0)		
SYMPTOMS					
Cough	168 (75.7)	104 (63.4)	16(4.0)	1.79 (1.15-2.79)	0.009
Expectoration	132 (59.5)	80 (48.8)	16 (4.0)	1.54 (1.02-2.31)	0.037
Nausea	70 (31.8)	36 (22.0)	18 (4.5)	1.65 (1.04-2.64)	0.033
Loss of appetite	137 (62.3)	84 (51.2)	18 (4.5)	1.57 (1.04-2.36)	0.030
SEVERITY ASSESSMENT					
CURB65 ≥3 *	23 (10.4)	30 (17.3)	8 (2.0)	0.55 (0.30-0.99)	0.047
VITAL PARAMETERS					
Oxygen saturation <96%	147 (64.2)	79 (46.0)	1 (0.2)	2.11 (1.40-3.15)	<0.001
Heart rate < 51 or >100 bpm/min	129 (56.3)	80 (46.2)	0 (0.0)	1.49 (1.00-2.23)	0.045
Fever >38ºC	64 (28.2)	30 (17.3)	2 (0.5)	1.87 (1.14-3.05)	0.011
BLOOD TESTS					
Leukocytes <3.5 or > 8.8 10E9/L	191 (83.4)	106 (61.3)	0 (0.0)	3.17 (1.99-5.04)	<0.001
Neutrophilocytes > 7.5 10E9/L	166 (73.1)	81 (47.6)	5 (1.2)	2.99 (1.96-4.55)	<0.001
Natrium <137 or > 145 mmol/L	114 (49.8)	55 (31.8)	0 (0.0)	2.12 (1.40-3.21)	<0.001
Bilirubin<5 or >25 mmol/L	32 (14.0)	37 (21.8)	4 (1.0)	0.58 (0.34-0.98)	0.045
C-Reactive Protein mg/L, n (%)			0 (0.0)		
<20 mg/L	15 (6.6)	59 (34.1)		1 (reference)	
21-99 mg/L	74 (32.3)	64 (37.0)		4.54 (2.35-8.78)	<0.001
≥ 100 mg/L	140 (61.1)	50 (28.9)		11.01(5.73-21.14)	<0.001

Statistically significant results from the unadjusted association between each candidate predictor and the outcome CAP.* CURB65: confusion, uremia, respiratory rate, blood pressure, age > 65 years.

Model development and performance

We developed a prediction model for diagnosing pneumonia in patients admitted with suspected infection (n=954) and compared it with the clinician's presumptive diagnosis. Supplementary table S3 presents the characteristics of the population randomised in the training and validation sets. The predictors associated with CAP in our final model are presented in Table 3.

Table 3: The complete diagnostic model, including the intercept

Intercept and predictors	ß Coefficient
Intercept	-1.66192
Dyspnea (yes)	0.35172
Expectoration (yes)	0.36250
Cough (yes)	0.39671
Common cold (yes)	0.34374
Malaise (yes)	0.07475
Chest pain (yes)	0.20499
Respiratory rate >20/min	0.14566
Oxygen saturation < 96%	0.24303
Abnormal auscultation findings (yes)	0.56758
Leucocytes*	0.00322
Neutrophilocytes**	0.08338
C-reactive protein <20 mg/L	-0.64269
Previous event of CAP (no)	-0.12006
Age of ≥ 75 and cough (yes)	0.53816
Age of ≥ 75 and oedema (no)	-0.05797
Age of ≥ 75 and glucose >11.0 mmol/L	0.88124
ROC AUC† (95% CI)	0.85 (0.77-0.92)

* Cut-off for leucocyttes: normal values 3.5 -8.8 10E9/L

**Neutrophilocytes: > 7.5 10E9/L

† ROC AUC = receiver-operating characteristic area under the curve

The model performance yielded an AUC of 0.85 (CI: 0.77-0.92) and the calibration of the model yielded p=0.227 after recalibration, demonstrating a good prediction of the proportion of CAP patients in the test sample (Supplementary figures S4 and S5).

Based on a lambda result of λ=0.0402856 and a probability threshold of 0.35, the LASSO calculation with characteristics predictive of CAP and the calculation of the final model with a cut-off value greater than 0 indicating the diagnosis CAP are presented in Supplemental material (Supplementary formulas S6 and S7).

At the optimal cut-off of 0.35, the prediction model yielded an 86.1% sensitivity and 64.1% specificity. Based on the trial population (Figure 1), the sensitivity of the prediction model was comparable to the initial diagnosis made by the ED physicians. However, the specificity and positive predictive value were significantly lower (Table 4).

Table 4: Performance of the predictive model compared to the initial diagnosis made by the ED physicians.

Performance	Sensitivity % (CI %)	Specificity % (CI %)	Positive predictive value % (CI %)	Negative predictive value % (CI %)
Predictive model	86.1 (79.1-93.1)	64.1 (57.1-71.1)	41.6 (34.6-48.6)	93.9 (86.9-100)
Physicians	86.4 (84.2-88.6)	74.9 (72.1-77.6)	57.0 (53.8-60.1)	93.5 (92.0-95.0)

The predictive model had a 35% cut-off and a prevalence of 22%. The prevalence of CAP was 28% in the population of 954 patients suspected of infection.

Model specification

The final model did not include the following possible predictors: lymphocytes, SARS-CoV-2, and BMI. The reasons were a high percentage of missings (lymphocytes 66.3%), clinical relevance, and statistical performance (BMI and SARS-CoV-2). These considerations are described in detail in Supplemental material.

DISCUSSION

More than every fourth patient with suspected infection was diagnosed with CAP (28%). The ED physicians suspected CAP in almost half (42%) of patients admitted with suspected infection. Patients with suspected CAP included 57% with a final expert diagnosis of CAP and 43% without CAP. We have identified twenty-seven clinical characteristics for patients diagnosed with CAP among those admitted suspected of infection. Patients with CAP were characterised by having more often a history of smoking, previous CAP, respiratory symptoms, abnormal lung auscultation, worse triage, and abnormal levels of infection biomarkers. Fewer clinic characteristics (thirteen) were identified for patients diagnosed with CAP among patients suspected of CAP by the ED physician and included typical respiratory symptoms but also gastrointestinal symptoms, abnormal vital signs, increased blood markers, and lower CURB-65 scores. The final diagnostic prediction model yielded thirteen diagnostic predictors for CAP recognised by the literature. The model performance was similar to the diagnosis made by the ED physicians regarding sensitivity and negative predictive value but not as good in determining the specificity and positive predictive values.

Our prediction model had a good performance (AUC 85%) and calibration (p=0.227), and with the best cut-off of 35%, the sensitivity reached 86.1% and specificity 64.1%. Therefore, the model could be tested externally and contribute to the initial management of CAP, guiding further clinical investigation. In this study, ED physicians who generally only had the patient's history and the results from a simple clinical examination diagnosed CAP with a comparable negative predictive value (93% vs. 94%) and a better positive predictive value (57% vs. 42%). Even though our model is not entirely comparable to the initial diagnosis made by the ED physicians due to the difference in the prevalence of CAP, our results are similar to a recent systematic review [43]. Other studies reported that ED physicians' accuracy in diagnosing CAP ranged from 76% to 96% [44], and artificial intelligence predicted the presence of pneumonia with a sensitivity of 94% and specificity of 50% [45]. These results show that there is room for improvement in diagnosing CAP. It could be achieved by including additional predictors such as biomarkers, e.g., procalcitonin, YKL-40, and surfactant protein-D [46, 47], molecular detection of respiratory pathogens [48], and/or improved imaging modalities [12, 14].

This prospective study highlights the challenges in identifying patients with CAP based on patient history, vital signs, and symptoms upon admission [20, 22, 46]. The initial CAP diagnosis often differs from the discharge diagnosis [10, 49]. A plausible cause for uncertainty in diagnosing CAP was the heterogenic presentation of symptoms overlapping with other diseases. We found that patients with verified CAP often had gastrointestinal symptoms, whereas patients not verified with CAP sometimes presented with typical respiratory symptoms and had more severe conditions measured by CURB-65. Typical respiratory symptoms could explain some CAP misclassification.

Misclassification of CAP may lead to unnecessary or ineffective antibiotic treatment, increased healthcare costs, delayed diagnosis, increased mortality, and increased risk of bacterial resistance [44, 50].

The predictors of CAP identified in this study are strongly represented in the literature [9, 20, 36, 37, 42, 46, 49]. Most prediction models for ED patients with CAP aim to predict prognostic outcomes such as disease severity and mortality [51]. Prior studies have investigated only a few diagnostic predictors or studied very selected patients [20, 22, 52]. The main reason for including several potential predictors and having age as a cross-factor in the development of our model was the expectation of finding predictors not represented in the literature and predictors specific for older patients (≥75 years). This is considered very relevant as the population worldwide ages [4, 16]. An age of ≥ 75 interacted with the symptoms of cough, blood glucose levels, and peripheral oedema. Peripheral oedema was associated with an absence of CAP where symptoms may be explained by other infections such as erysipelas or cardiac heart failure patients with respiratory symptoms. In addition, hyperglycemia has been recognized as a predictor associated with poorer patient outcomes for elderly CAP patients, regardless of their history of diabetes [53, 54].

Even though the literature highlights malnutrition as a strong prognostic predictor for CAP [33, 35, 55], we excluded BMI from our final model. Measuring weight and height is not a priority in acute settings where vital parameters, symptoms, and point-of-care biomarkers are the primary observations in the diagnostic process. Another concern was that BMI was missing in 26.3% of the population, and bias may arise due to systematic differences between subjects with complete datasets and subjects with

missing data. Patients with missing BMI data may be more frail, incapable, or difficult to transfer. A model including BMI could be a better choice in a primary care setting, where patients are not necessarily as acutely ill and may be able to weigh themselves.

A major strength of this study is the completeness of data from medical charts and patient interviews combined with CAP diagnoses assigned by a panel of experts. The experts had a range of information from the patient's medical records, including chest x-ray, chest CT for patients suspected of CAP, and microbiology results available for many of the patients. In addition, to identifying possible predictors, we included many relevant and easily accessible clinical parameters. Finally, we excluded patients infected with SARS-CoV-2 from the study to increase the potential generalisability for CAP patients after the pandemic.

This study also has several limitations. Multiple testing and mass significance are potentially a problem in this study. Methods, such as Bonferroni-Holm correction, could have been applied to counteract this problem [56]. However, the univariate analyses were conducted for exploratory and descriptive purposes only. Therefore, these results should be interpreted cautiously, and the findings should be used as hypothesis-generating rather than conclusive. Another concern is that even though the reference standard of CAP was the same for the model performance and the initial diagnosis of the ED physicians, the expert panel might have a better prerequisite to diagnose CAP in suspected CAP patients due to the availability of results from imaging and microbiological tests, and better register of patient’s symptoms. It might lead to differential verification bias overestimating the ED physician's accuracy in

diagnosing CAP [57]. This assumption may be supported by the higher specificity of CAP diagnoses from ED physicians.

Another limitation is the selected population of the patients allocated to the internal medicine specialty that may have masked atypical predictors from patients assigned to other specialities. Furthermore, some patients with atypical clinical presentation might have an infection that the ED physician had not suspected upon admission and therefore was not included in our study. Patients with severe condition or acute cognitive impairment who could not consent were excluded. A broader patient inclusion may contribute to a model that identifies other predictors assisting in diagnosing CAP as the clinical presentation might differ from those admitted with suspected CAP and capable of consent. Another limitation of the development of the model, was the choice of cut-offs for blood tests routinely used in our institutions, this pragmatic choice reflects our clinical practice. However, it does raise questions about the applicability in other settings that apply different cut-offs.

This population cohort could be applicable as a test validation cohort for future models as the data collection of these well-known predictors of CAP is reproducible across EDs. The development of automatic extraction for a prediction model from electronic medical records using artificial intelligence could be of great value in a busy ED. In conclusion, typical respiratory symptoms combined with abnormal vital signs and elevated infection biomarkers are predictors for CAP upon admission to an ED. A diagnostic prediction model based on these predictors is of limited value. Future prediction models should include novel diagnostic tools, imaging, PCR analysis, and/or serological markers not routinely used in clinical practice to

improve model performance, helping diagnose CAP more accurately at the ED.

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Authors' contributions: MBC, FSR, CBM, TS, HSA, MHL, AH, and MAH were involved in the study's design. MBC performed the literature search and drafted the original work. MBC, MHL, AH, MAH, JJS, and FK recruited patients and collected data. CBM and MAH participated in the expert panel. HSA was the study investigator-, and coordinated and supervised the project. MBC performed the statistical analyses. CBM was the chief research officer responsible for supervising the overall study. All authors, MBC, FSR, CBM, TS, HSA, MHL, AH, MAH, FK, and JJS critically revised and approved the final manuscript.

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Availability of data and materials: Due to Danish laws on personal data, data cannot be shared publicly. To request data, please contact the corresponding author for more information. The person responsible for the research was the principal investigator and corresponding author (MBC) in collaboration with the University Hospital of Southern Denmark. This organization owns the data and can provide access to the final data set.

REFERENCES

1. Søgaard M, Nielsen RB, Schønheyder HC, Nørgaard M, Thomsen RW. Nationwide trends in pneumonia hospitalization rates and mortality, Denmark 1997-2011. Respir Med. 2014;108(8):1214-22.doi:10.1016/j.rmed.2014.05.004

2. McLaughlin JM, Khan FL, Thoburn EA, Isturiz RE, Swerdlow DL. Rates of hospitalization for community-acquired pneumonia among US adults: A systematic review. Vaccine. 2020;38(4):741-51.doi:10.1016/j.vaccine.2019.10.101

3. Welte T, Torres A, Nathwani D. Clinical and economic burden of community-acquired pneumonia among adults in Europe. Thorax. 2012;67(1):71-9

4. Laporte L, Hermetet C, Jouan Y, Gaborit C, Rouve E, Shea KM, et al. Ten-year trends in intensive care admissions for respiratory infections in the elderly. Ann Intensive Care. 2018;8(1):84.doi:10.1186/s13613-018-0430-6

5. GBD 2016 Lower Respiratory Infections Collaborators. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Infect Dis. 2018;18(11):1191-210.doi:10.1016/s1473-3099(18)30310-4

6. Meehan TP, Fine MJ, Krumholz HM, Scinto JD, Galusha DH, Mockalis JT, et al. Quality of care, process, and outcomes in elderly patients with pneumonia. JAMA. 1997;278(23):2080-4.doi:10.1001/jama.1997.03550230056037

7. Bell BG, Schellevis F, Stobberingh E, Goossens H, Pringle M. A systematic review and meta-analysis of the effects of antibiotic consumption on antibiotic resistance. BMC Infect Dis. 2014;14 (1):13.doi:10.1186/1471-2334-14-13

8. Metlay JP, Waterer GW, Long AC, Anzueto A, Brozek J, Crothers K, et al. Diagnosis and treatment of adults with community-acquired pneumonia. An official clinical practice guideline of the American Thoracic Society and

Infectious Diseases Society of America. Am J Respir Crit Care Med. 2019;200(7):e45-e67.doi:10.1164/rccm.201908-1581ST

9. Musher DM, Thorner AR. Community-acquired pneumonia. N Engl J Med. 2014;371(17):1619-28.doi:10.1056/NEJMra1312885

10. Chandra A, Nicks B, Maniago E, Nouh A, Limkakeng A. A multicenter analysis of the ED diagnosis of pneumonia. Am J Emerg Med. 2010;28(8):862-5.doi:10.1016/j.ajem.2009.04.014

11. Claessens YE, Debray MP, Tubach F, Brun AL, Rammaert B, Hausfater P, et al. Early chest computed tomography scan to assist diagnosis and guide treatment decision for suspected community-acquired pneumonia. Am J Respir Crit Care Med. 2015;192(8):974-82.doi:10.1164/rccm.201501-0017OC

12. Ye X, Xiao H, Chen B, Zhang S. Accuracy of Lung Ultrasonography versus Chest Radiography for the Diagnosis of Adult Community-Acquired Pneumonia: Review of the Literature and Meta-Analysis. PLoS One. 2015;10(6):e0130066.doi:10.1371/journal.pone.0130066

13. Kamat IS, Ramachandran V, Eswaran H, Guffey D, Musher DM. Procalcitonin to distinguish viral from bacterial pneumonia: a systematic review and meta-analysis. Clin Infect Dis. 2020;70(3):538-42.doi:10.1093/cid/ciz545

14. Gentilotti E, De Nardo P, Cremonini E, Górska A, Mazzaferri F, Canziani LM, et al. Diagnostic accuracy of point-of-care tests in acute community-acquired lower respiratory tract infections. A systematic review and meta-analysis. Clin Microbiol Infect. 2022;28(1):13-22.doi:10.1016/j.cmi.2021.09.025

15. Welker JA, Huston M, McCue JD. Antibiotic timing and errors in diagnosing pneumonia. Arch Intern Med. 2008;168(4):351-6.doi:10.1001/archinternmed.2007.84

16. World Health Organization. Aging and Health [Internet]. 2022 October 1 [cited 2022 October 28]. Available from: https://www.who.int/news-room/fact-sheets/detail/ageing-and-health.

17. Weir DL, Majumdar SR, McAlister FA, Marrie TJ, Eurich DT. The impact of multimorbidity on short-term events in patients with community-

acquired pneumonia: prospective cohort study. Clin Microbiol Infect. 2015;21(3):264.e7-.e13.doi:10.1016/j.cmi.2014.11.002

18. Sakakibara T, Shindo Y, Kobayashi D, Sano M, Okumura J, Murakami Y, et al. A prediction rule for severe adverse events in all inpatients with community-acquired pneumonia: a multicenter observational study. BMC Pulm Med. 2022;22(1):34.doi:https://dx.doi.org/10.1186/s12890-022-01819-0

19. Gong L, He D, Huang D, Wu Z, Shi Y, Liang Z. Clinical profile analysis and nomogram for predicting in-hospital mortality among elderly severe community-acquired pneumonia patients with comorbid cardiovascular disease: a retrospective cohort study. BMC Pulm Med. 2022;22(1):312.doi:10.1186/s12890-022-02113-9

20. Ding F, Han L, Yin D, Zhou Y, Ji Y, Zhang P, et al. Development and validation of a simple tool composed of items on dyspnea, respiration rates, and C-reactive protein for pneumonia prediction among acute febrile respiratory illness patients in primary care settings. BMC Med. 2022;20(1):360.doi:10.1186/s12916-022-02552-5

21. Hammond A, Halliday A, Thornton HV, Hay AD. Predisposing factors to acquisition of acute respiratory tract infections in the community: a systematic review and meta-analysis. BMC Infect Dis. 2021;21(1):1254.doi:10.1186/s12879-021-06954-3

22. Ebell MH, Chupp H, Cai X, Bentivegna M, Kearney M. Accuracy of signs and symptoms for the diagnosis of community-acquired pneumonia: a meta-analysis. Acad Emerg Med. 2020;27(7):541-53.doi:10.1111/acem.13965

23. Kitazawa T, Yoshihara H, Seo K, Yoshino Y, Ota Y. Characteristics of pneumonia with negative chest radiography in cases confirmed by computed tomography. J Community Hosp Intern Med Perspect. 2020;10(1):19-24.doi:10.1080/20009666.2020.1711639

24. Moons KG, Altman DG, Reitsma JB, Ioannidis JP, Macaskill P, Steyerberg EW, et al. Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): explanation and elaboration. Ann Intern Med. 2015;162(1):W1-W73.doi:10.7326/m14-0698

25. Skjøt-Arkil H, Heltborg A, Lorentzen MH, Cartuliares MB, Hertz MA, Graumann O, et al. Improved diagnostics of infectious diseases in emergency departments: a protocol of a multifaceted multicentre diagnostic study. BMJ Open. 2021;11(9):e049606.doi:10.1136/bmjopen-2021-049606

26. Nørgaard B, Mogensen CB, Teglbjærg LS, Brabrand M, Lassen AT. Diagnostic packages can be assigned accurately in emergency departments. A multi-centre cohort study. Dan Med J. 2016;63(6)

27. Harris PA, Taylor R, Minor BL, Elliott V, Fernandez M, O'Neal L, et al. The REDCap consortium: building an international community of software platform partners. J Biomed Inform. 2019;95:103208.doi:10.1016/j.jbi.2019.103208

28. Mutepe ND, Cockeran R, Steel HC, Theron AJ, Mitchell TJ, Feldman C, et al. Effects of cigarette smoke condensate on pneumococcal biofilm formation and pneumolysin. Eur Respir J. 2013;41(2):392-5.doi:10.1183/09031936.00213211

29. Samokhvalov AV, Irving HM, Rehm J. Alcohol consumption as a risk factor for pneumonia: a systematic review and meta-analysis. Epidemiol Infect. 2010;138(12):1789-95.doi:10.1017/s0950268810000774

30. Torres A, Peetermans WE, Viegi G, Blasi F. Risk factors for community-acquired pneumonia in adults in Europe: a literature review. Thorax. 2013;68(11):1057-65.doi:10.1136/thoraxjnl-2013-204282

31. Barbagelata E, Cillóniz C, Dominedò C, Torres A, Nicolini A, Solidoro P. Gender differences in community-acquired pneumonia. Minerva Med. 2020;111(2):153-65.doi:10.23736/s0026-4806.20.06448-4

32. Baik I, Curhan GC, Rimm EB, Bendich A, Willett WC, Fawzi WW. A prospective study of age and lifestyle factors in relation to community-acquired pneumonia in US men and women. Arch Intern Med. 2000;160(20):3082-8.doi:10.1001/archinte.160.20.3082

33. Cillóniz C, Dominedò C, Pericàs JM, Rodriguez-Hurtado D, Torres A. Community-acquired pneumonia in critically ill very old patients: a growing problem. Eur Respir Rev. 2020;29(155):190126.doi:10.1183/16000617.0126-2019

34. Reisinger EC, Fritzsche C, Krause R, Krejs GJ. Diarrhea caused by primarily non-gastrointestinal infections. Nat Clin Pract Gastroenterol Hepatol. 2005;2(5):216-22

35. Riquelme OR, Riquelme OM, Rioseco ZML, Gómez MV, Cárdenas G, Torres C. Neumonía adquirida en la comunidad en el anciano hospitalizado: Aspectos clínicos y nutricionales. [Community-acquired pneumonia in the elderly: clinical and nutritional aspects]. Rev Med Chil. 2008;136(5):587-93.doi:10.4067/S0034-98872008000500006

36. Moore M, Stuart B, Little P, Smith S, Thompson MJ, Knox K, et al. Predictors of pneumonia in lower respiratory tract infections: 3C prospective cough complication cohort study. Eur Respir J. 2017;50(5).doi:10.1183/13993003.00434-2017

37. van Vugt SF, Broekhuizen BD, Lammens C, Zuithoff NP, de Jong PA, Coenen S, et al. Use of serum C reactive protein and procalcitonin concentrations in addition to symptoms and signs to predict pneumonia in patients presenting to primary care with acute cough: diagnostic study. BMJ. 2013;346:f2450.doi:10.1136/bmj.f2450

38. Alam N, Vegting IL, Houben E, van Berkel B, Vaughan L, Kramer MH, et al. Exploring the performance of the National Early Warning Score (NEWS) in a European emergency department. Resuscitation. 2015;90:111-5.doi:10.1016/j.resuscitation.2015.02.011

39. Plesner LL, Iversen AKS, Langkjær S, Nielsen TL, Østervig R, Warming PE, et al. The formation and design of the TRIAGE study-baseline data on 6005 consecutive patients admitted to hospital from the emergency department. Scand J Trauma Resusc Emerg Med. 2015;23:106.doi:10.1186/s13049-015-0184-1

40. Ravioli S, Germann C, Gygli R, Exadaktylos AK, Lindner G. Age- and sex-related differences in community-acquired pneumonia at presentation to the emergency department: a retrospective cohort study. Eur J Emerg Med. 2022;29(5):366-72.doi:10.1097/mej.0000000000000933

41. Akhtar A, Hassali MAA, Zainal H, Ali I, Iqbal MS, Khan AH. Respiratory-tract infections among geriatrics: prevalence and factors associated with the treatment outcomes. Ther Adv Respir Dis. 2021;15:1753466620971141.doi:10.1177/1753466620971141

42. Metlay JP, Schulz R, Li YH, Singer DE, Marrie TJ, Coley CM, et al. Influence of age on symptoms at presentation in patients with community-acquired pneumonia. Arch Intern Med. 1997;157(13):1453-9.doi:doi:10.1001/archinte.1997.00440340089009

43. Dale AP, Marchello C, Ebell MH. Clinical gestalt to diagnose pneumonia, sinusitis, and pharyngitis: a meta-analysis. Br J Gen Pract. 2019;69(684):e444-e53.doi:10.3399/bjgp19X704297

44. Ray P, Birolleau S, Lefort Y, Becquemin MH, Beigelman C, Isnard R, et al. Acute respiratory failure in the elderly: etiology, emergency diagnosis and prognosis. Crit Care. 2006;10(3):R82.doi:10.1186/cc4926

45. Heckerling PS, Gerber BS, Tape TG, Wigton RS. Prediction of community-acquired pneumonia using artificial neural networks. Med Decis Making. 2003;23(2):112-21.doi:10.1177/0272989x03251247

46. Htun TP, Sun Y, Chua HL, Pang J. Clinical features for diagnosis of pneumonia among adults in primary care setting: A systematic and meta-review. Sci Rep. 2019;9(1):7600.doi:10.1038/s41598-019-44145-y

47. Spoorenberg SM, Vestjens SM, Rijkers GT, Meek B, van Moorsel CH, Grutters JC, et al. YKL-40, CCL18 and SP-D predict mortality in patients hospitalized with community-acquired pneumonia. Respirology. 2017;22(3):542-50.doi:10.1111/resp.12924

48. Gastli N, Loubinoux J, Daragon M, Lavigne JP, Saint-Sardos P, Pailhoriès H, et al. Multicentric evaluation of BioFire FilmArray Pneumonia Panel for rapid bacteriological documentation of pneumonia. Clin Microbiol Infect. 2021;27(9):1308-14.doi:10.1016/j.cmi.2020.11.014

49. Metlay JP, Fine MJ. Testing strategies in the initial management of patients with community-acquired pneumonia. Ann Intern Med. 2003;138(2):109-18.doi:10.7326/0003-4819-138-2-200301210-00012

50. Kanwar M, Brar N, Khatib R, Fakih MG. Misdiagnosis of community-acquired pneumonia and inappropriate utilization of antibiotics: side effects of the 4-h antibiotic administration rule. Chest. 2007;131(6):1865-9.doi:10.1378/chest.07-0164

51. Loke YK, Kwok CS, Niruban A, Myint PK. Value of severity scales in predicting mortality from community-acquired pneumonia: systematic review and meta-analysis. Thorax. 2010;65(10):884-90.doi:10.1136/thx.2009.134072

52. Okimoto N, Yamato K, Kurihara T, Honda Y, Osaki K, Asaoka N, et al. Clinical predictors for the detection of community-acquired pneumonia in adults as a guide to ordering chest radiographs. Respirology. 2006;11(3):322-4.doi:10.1111/j.1440-1843.2006.00846.x

53. Zeng W, Huang X, Luo W, Chen M. Association of admission blood glucose level and clinical outcomes in elderly community-acquired pneumonia patients with or without diabetes. Clin Respir J. 2022;16(8):562-71.doi:10.1111/crj.13526

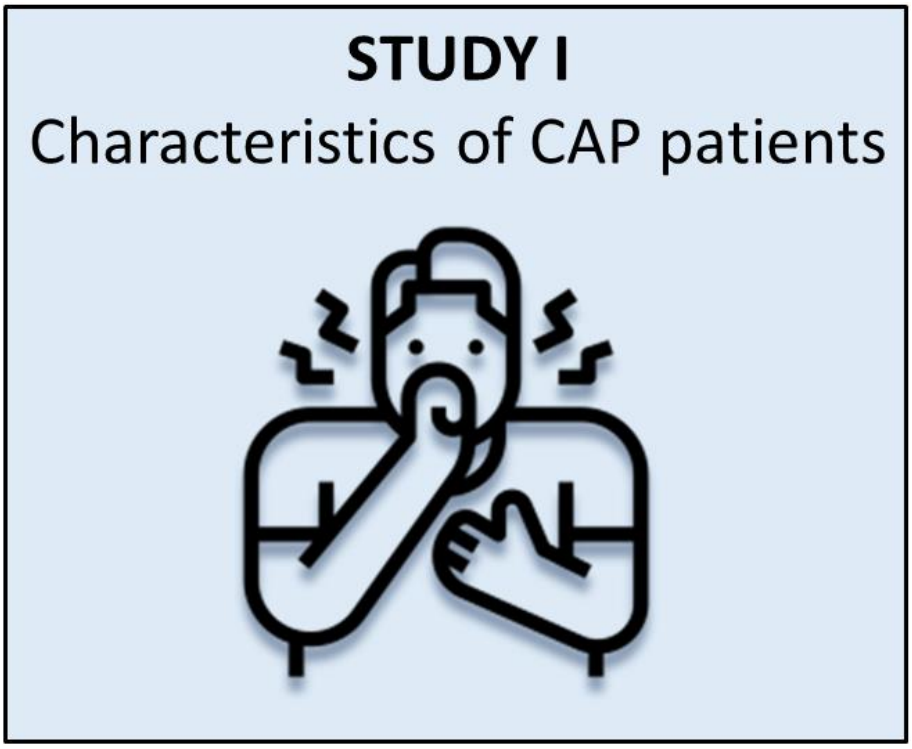
54. Barmanray RD, Cheuk N, Fourlanos S, Greenberg PB, Colman PG, Worth LJ. In-hospital hyperglycemia but not diabetes mellitus alone is associated with increased in-hospital mortality in community-acquired pneumonia (CAP): a systematic review and meta-analysis of observational studies prior to COVID-19. BMJ Open Diabetes Res Care. 2022;10(4).doi:10.1136/bmjdrc-2022-002880

55. Yeo HJ, Byun KS, Han J, Kim JH, Lee SE, Yoon SH, et al. Prognostic significance of malnutrition for long-term mortality in community-acquired pneumonia: a propensity score matched analysis. Korean J Intern Med. 2019;34(4):841-9.doi:10.3904/kjim.2018.037

56. Sedgwick P. Multiple significance tests: the Bonferroni correction. BMJ. 2012;344

57. Rutjes AW, Reitsma JB, Di Nisio M, Smidt N, van Rijn JC, Bossuyt PM. Evidence of bias and variation in diagnostic accuracy studies. CMAJ. 2006;174(4):469-76.doi:10.1503/cmaj.050090

11.1.1 Paper I – Supplementary material



Community-acquired pneumonia – Use of clinical characteristics of acutely admitted patients for the development of a diagnostic model: A cross-sectional multicentre study

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Table of Contents

Table S1: Description of the 70 pre-specified predictors for CAP

Source: *The patient interview*			
Group	Variable name	Measurement	Consideration/assumption Considerations to collect data from these predictors were based on the described literature and expert consensus together with the project group
Demographic information	Age	Continuous, years	Age is a risk factor for CAP [1]. Several studies stratify age groups when investigating pneumonia due to several atypical symptoms and signs and the absence of respiratory symptoms among the elderly. Stratified age groups differ in cut-offs between the ages of ≥65 to ≥80 years old [2-7].
	Gender	Binary 1=Male 0=Female	The risk of CAP is higher for males [8].CAP is more severe [7] leading to higher mortality in males [9]. Males' lifestyle factors differ from women resulting in a higher risk of CAP [10].
	Civil status (Living alone)	Binary (Yes/no)	Living alone has a two-fold association with having one or more respiratory tract infections [11].
	Nursing home residence	Binary (Yes/no)	Nursing home residents were found to have several comorbidities [12] and lower physical functioning levels, which might result in a higher risk of CAP [13].
	Employment	Categorical: 1=Working 2=Retired 0=Others (e.g. students, flex job)	Low income and unemployment are associated with readmissions after CAP [14].
Symptoms	Feeling unwell/ Malaise	Binary (Yes/No) Symptoms within 14 days prior to ED admission.	Malaise has been identified as one of the most frequent symptoms for patients infected with *Mycoplasma pneumoniae*[15].
	Fatigue		Fatigue is associated with pneumonia especially in elderly patients [4].
	Headache		Headache is one of the clinical findings of symptoms of CAP [7, 15]. However, headaches were less common in the older population [7].
	Dizziness		The rationale of the presence of dizziness as a symptom relied on the assumption that several factors such as polypharmacy[16], combined with comorbidities such as cardiovascular diseases [17], symptoms such as confusion, conditions of frailty and malnutrition [18], and lower oxygen saturation [19]could contribute to dizziness.

	Confusion		Confusion e.g. altered mental status or delirium was significantly more frequent in CAP patients [2, 4].
	Dyspnea		Dyspnea was identified as a strong prediction of CAP among febrile patients [20] and one of the main symptoms of pneumonia [2, 21].
	Cough		Cough is a common symptom and one of the most frequent increasing the likelihood of detecting a viral pathogen among CAP patients [15, 22]. Algorithms included cough as a diagnostic predictor [23], and dry cough was a strong predictor in a prediction model for *Legionella pneumoniae* [24]. Cough was less common in older population [7].
	Secretions		Purulent secretions were a significant symptom and predictor for CAP patients [20, 21].
	Sore throat		Some studies identified sore throat as a symptom of CAP [15], and one included the symptom in the prediction rules of pneumonia [5].
	Cold		Among respiratory diseases, the common cold is one of the most frequent, with symptoms similar to CAP [25].
	Fever feeling		Quantified from reported chills or night sweat or fever measured at home. Included as a rationale of fever.
	Chest pain		Chest pain has been used as a single predictor of CAP [18, 20, 23] or a combined diagnostic predictor [23] and may present as a secondary symptom of coughing or pleuritic involvement [26]. However, chest pain was less common in the older population [7].
	Peripheral edema		The rationale for including peripheral edema as possible predictor is that it is included in the clinical assessment at admission. In case of peripheral edema and respiratory symptoms of dyspnea, chest pain and a history of cardiovascular disease, CAP could be ruled out as a tentative diagnosis replaced with suspicion of cardiovascular disease.
	Nausea		Gastrointestinal symptoms such as nausea, vomiting and diarrhea manifests in 20% of the CAP population [26].
	Vomiting		Gastrointestinal symptoms such as nausea, vomiting and diarrhea manifests in 20% of the CAP population [26].
	Loss of appetite		Loss of appetite could be present in the case of gastrointestinal symptoms [26] and could result from malnutrition [18].
	Abdominal pain		Abdominal pain may be present in the case of gastrointestinal symptoms

			described above and, therefore, is included in the model [26].
	Diarrhea		Gastrointestinal symptoms such as nausea, vomiting and diarrhea manifests in 20% of the CAP population [26].
	Pain in muscles and joints including back pain		Muscle and joint pain are associated with viral pneumonia as influenza, especially among younger patients and therefore is included in our model [27].
Previous event of CAP	Previous event of CAP	Categorical: 0= Never 1= Once 2= More than once	A previous diagnosis of CAP was reported as having robust evidence as a risk factor for CAP [1]. Furthermore, any hospitalization in the previous five years was reported as a predisposing factor for CAP [8].
Lifestyle factors and aids	Smoke	Categorical: 0=Never been a smoker 1=Current smoker 2=Previous smoker	Smoking has been associated with an increased risk of CAP in several studies [1, 8, 10, 17], and has a strong association with the treatment outcomes of elderly individuals with respiratory tract infections [28].
	Alcohol	Doses per week (a dose=12 grams (1, 5 cl) alcohol). Categories based on the Danish Board of Health recommendations [29]. 0=No alcohol 1=1-7 doses/week maximum doses recommended for women 2=8-14 doses/week maximum dose recommended for men 3= >14 doses	Alcohol has also been associated with increased CAP risk and with treatment outcomes. The risk increases in individuals with higher consumption (>41 g/day) compared to those who consume no alcohol [10, 17, 28].
	Physical activity levels	We categorized physical activity levels based on recommendations from the world health organization for adults with a minimum 150 min/week [30]. 1= Not physically active 2= Less than 2.5hrs/week 3= More than 2.5hrs/week	The risk of CAP decreased in physically active women [10]. In addition, a high level of activity protects against upper respiratory tract infections and reduces the severity and symptoms of the infection [13].
	Activities of daily living	Binary (yes/no) Yes= If the patient had one or more dependencies regarding:	Difficulty in maintaining toilet hygiene, preparing meals, and being unable to transfer were associated with an increased risk of respiratory infections [31].

		bathing, dressing, toileting, transfer, continence and feeding.	
Source: *Variables extracted from the patient's medical report*			
Comorbidities (diseases)	Neurological	Binary (Yes/no) If the patient was diagnosed with one of these diagnoses.	Cerebrovascular disease/stroke and Parkinson's disease approximately doubled the risk of CAP [17].
	Pulmonary		A history of pneumonia increased the risk of a subsequent episode and patients with chronic respiratory diseases, including chronic obstructive pulmonary disease, bronchitis or asthma, had up to a fourfold increase in the risk of CAP [1, 4, 17].
	Endocrinological		Chronic liver conditions were reported as a risk factor of CAP [8]. Recently, diabetes mellitus has been described as an independent risk factor for sepsis secondary to CAP in very old patients [4] and data from several studies showed an association between diabetes mellitus and moderate risk of CAP [17].
	Renal		Chronic renal disease was reported as an independent risk factor for sepsis secondary to CAP in very old patients [4, 8] and chronic renal disease increased the risk of CAP twofold [17].
	Cardiovascular		Chronic cardiovascular disease increased the risk of CAP up to threefold [4, 17].
	Gastrointestinal		The rationale for including gastrointestinal diseases in the model was that CAP patients have gastrointestinal symptoms that could be related to a differential diagnosis besides CAP.
	Dementia		Dementia approximately doubles the risk of CAP [17].
	Cancer		Cancer was associated with a moderate increase in CAP risk, and a single study reported a fivefold increased risk of CAP for patients with lung cancer [17].
	Rheumatological		A moderate risk of CAP was found in patients with rheumatological diseases [17].
Pharmacological treatments	Polypharmacy	Binary (yes/no) Regular consumption of at least five medications	The increased number of comorbidities of older patients increases the risk of polypharmacy [4, 32]. The prevalence of polypharmacy reached almost 40% among individuals with respiratory tract infections above age 65 years and had a twofold association with treatment outcomes of respiratory tract infections [28]. Furthermore, the prevalence of polypharmacy increased from 45% to 74%, irrespective of antibiotic use if

			patients were hospitalized with CAP [16].
	Analgesics	Binary (Yes/no) Regular consumption of analgesics	A systematic review reported an association between prescribed opioids and CAP [33].
	Vaccination SARS-CoV-2	Binary (Yes/no) Recent vaccination for SARS-CoV-2	SARS-CoV-2 vaccination was reported during the clinical assessment but was taken out of the model, as the model would be used after the pandemic when vaccination for SARS-CoV-2 rates might decrease. However, the inclusion of this variable did not change the final predictive model.
	Vaccination pneumococcus	Binary (Yes/no) Pneumococcus vaccine (not specified) within 5 years	*Streptococcus pneumoniae* is one of the most causative pathogens of CAP and the vaccine could be a possible protective predictor for CAP as the risk of CAP increases among those unvaccinated [1, 34, 35].
	Vaccination influenza	Binary (Yes/no) Season influenza vaccine 2020/2021	Influenza vaccine can reduce hospitalization but is questionable if it could have a protective effect in admitted patients [1, 36], therefore, we included this possible predictor to investigate if it could have a protective role in our population.
Severity assessment	CURB-65	Binary ≥ 3 points (Yes/no) Definition: Confusion, urea >7 mmol/L, respiratory rate ≥ 30 bpm, blood pressure (≤90 for systolic blood pressure or ≤60 for diastolic blood pressure, age > 65 years) Score: one point for each present variable. CURB65≥ 3= severe condition	CURB65 is an assessment tool for the severity of CAP [37] recommended by the guidelines in Europe [38] including in Denmark [39].
	Triage	Based on the 5-level triage system "Danish emergency department triage" (DEPT) [40, 41], we categorized the following: Red/Orange and Green/Blue were pooled due to few patients in the blue and red groups: 1= Red/Orange 2= Yellow 3= Green/Blue	DEPT is a Danish adaption and modification of the "Adaptive Process Triage" (ADAPT) developed in Sweden [42]. DEPT was chosen as it is routinely used in the three included sites. Furthermore, in Denmark, most EDs have implemented formalized triage called "Danish Emergency Process Triage". DEPT shares core similarities with widespread standardized 5-level triage systems [43].

Vital parameters All vital parameters regardless of diastolic blood pressure were based on The National Early Warning Score (NEWS) [44]. This score was chosen as it is routinely used in the three EDs included in this study and cut-offs values in predicting CAP are similar from the literature.	Oxygen saturation	Binary < 96 % (Yes/no) The cut-off was based on The National Early Warning Score (NEWS) [44]. However, we did not differentiate between patients with chronic obstructive pulmonary disease.	A similar cut-off of oxygen saturation has been used in investigating predictors for CAP [19].
	Heart rate	Binary < 51 or >90 bpm (Yes/no)	Some studies have investigated and pointed out that a higher heart rate with similar cut-offs as a predictor for CAP [19, 45, 46].
	Blood pressure systolic	Binary <111 or >219 mmHg (Yes/no)	Other cut-offs based on the CURB65-score or lower level of triage (<90mmHg) have been used to predict a high risk of adverse events among inpatients with CAP [47]. This cut-off was also explored in our model without resulting in any difference.
	Blood pressure diastolic	Binary ≤60 mmHg (Yes/no) Based on severity assessment CURB65-score [37]. The NEWS does not include diastolic blood pressure and therefore the value from CURB-65 was chosen.	CURB-65 is routinely used in Denmark as a severity score and is included in the guidelines for antibiotic treatment [39]. As systolic blood pressure has been investigated in prediction rules, we added diastolic blood pressure to our model to explore this variable as a predictor for CAP.
	Respiratory rate (RR)	Binary >20 breaths/min (Yes/no)	There are different cut-offs of RR in the literature [20, 47]. RR> 20/min was defined as a strong prediction of CAP among febrile patients [20].
	Temperature	Binary >38 ºC (Yes/no) Measured with ear thermometer [48].	Different cut-offs have been investigated, including the cut-off of >38ºC used in this study [49]. Independent of cut-offs, several studies have identified fever as a predictor of CAP [19-21, 23, 45]. However, fever is less common and generally absent in the older population [7].
	Glascow coma score	Binary >15 (Yes/no)	Cognitive impairment [32] has been reported as a strong risk factor for delirium and confusion as a predictor of the severity of CAP [47]. Altered mental status is associated with CAP, especially in the elderly [18].
Blood tests The literature does not describe a clear cut-off for the diagnosis of CAP.	Hematocrit	Hematocrit (%), median (IQR) Binary (Yes/no) Cut-off: 40-50 for males	A hematocrit value of less than 35% was an independent predictor for severity and 2 years of mortality ($p = 0.035$) [50].

We chose a pragmatic approach and applied the cut-offs of serum biomarkers used in the EDs from our institution to reflect reality. Most of the serological biomarkers have been studied for prognostic purposes. We have included these as potential predictors for CAP to investigate their diagnostic prediction performance combined with signs and symptoms. Binary (Yes/no) measures. Yes= abnormal/ outside of the cut-off No= normal/ within the cut-off		and 35-46 for females Yes= outside of the cut-off No= within the cut-off	
	Hemoglobin	Hemoglobin mmol/L, median (IQR) Binary (Yes/no) Cut-off: 8.3-10.5 for males and 7.3-9.5 for females Yes= outside of the cut-off No= within the cut-off	Hemoglobin correlates with frailty in the elderly and indirectly could be a predictor that should be investigated [51].
	Leukocytes	Leukocytes 10E9/L, median (IQR) Binary (Yes/no) Cut-off: 3.5-8.8 Yes= outside of the cut-off No= within the cut-off	Elevated leucocytes have been reported as a predictor for CAP, especially in pneumonia with negative chest x-ray [52].
	Platelets	Platelets 10E9/L, median (IQR) Binary (Yes/no) Cut-off: 145-350 for males and 165-400 for females Yes= outside of the cut-off No= within the cut-off	Platelet count < 171 × 109/L was included in a prediction model for *legionella pneumoniae* showing a high diagnostic accuracy [AUC 0.89 (95% CI 0.86–0.93)] [24].
	Neutrophils	Neutrophilocytes 10E9/L, median (IQR) Binary (Yes/no) Cut-off: > 7.5 Yes= >7.5 No= ≤ 7.5	The neutrophil to lymphocyte ratio had a high diagnostic value for CAP patients [53]. Furthermore, higher mortality risk was found for CAP patients and if measured in the early stage of CAP could contribute to the diagnostic and disease severity [54].
	Lymphocytes	Lymphocytes 10E9/L, median (IQR) Binary (Yes/no) Cut-of: 1.00-4.00 Yes= outside of the cut-off No= within the cut-off	The neutrophil to lymphocyte ratio has been studied in prognostic studies and is associated with higher mortality risk in CAP patients and if measured in the early stage of CAP could contribute to the diagnostic and disease severity [54].
	Albumin	Albumin g/L, median (IQR) Binary (Yes/no) Cut-off: 34-45	The ratio of blood urea and albumin has been investigated as a predictive factor for CAP, but poor model performance advocated for further investigation [55]. Furthermore, albumin correlates with

		Yes= outside of the cut-off No= within the cut-off	frailty in the elderly and indirectly could be a predictor that should be investigated as frailty has been associated with an increased risk of CAP [51]. In addition, serum albumin (<3.4 g/dl) was associated with higher mortality for elderly patients with CAP [18] and was included in a prediction rule for severe adverse events in patients hospitalized with CAP (< 2 g/dL, 2 points; 2–3 g/dL, 1 point) [47].
	Creatinine	Creatinine μmol/L, median (IQR) Binary (Yes/no) Cut-off: 60-105 for males and 45-90 for females Yes= outside of the cut-off No= within the cut-off	Elevated creatinine levels have been reported with almost a sixfold association of poor CAP outcome (OR=5.67; 95%CI: 1.72-18.65) [56]. This result is supported by another study that showed that serum creatinine levels of ≥ 2.8 were a strong predictor of in-hospital mortality in adults with CAP when compared with five serum biomarkers [57].
	Blood urea	Blood urea nitrogen mmol/L, median (IQR) Binary (Yes/no) Cut-off: 3-5-8.1 for males and 3.1-7.9 for females Yes= outside of the cut-off No= within the cut-off	The ratio of blood urea and albumin has been investigated as a predictive factor for CAP, but poor model performance advocated for further investigation [55].
	Natrium	Natrium mmol/L, median (IQR) Binary (Yes/no) Cut-off: 137-145 Yes= outside of the cut-off No= within the cut-off	Hyponatremia < 133 mmol/L was one of the strong predictors in the prediction of CAP caused by *legionella pneumoniae* [24].
	Prothrombin time-international normalized ratio	Prothrombin (IQR) Binary (Yes/no) Cut-off: <1.2 Yes= ≥ 1.2 No= <1.2	Prothrombin time-international normalized ratio was investigated to distinguish Influenza A (H1N1) from other pneumonia. Prothrombin times were lower in H1N1 compared with non-H1N1 pneumonia patients (p=0.04) [58]. Furthermore, it has been investigated as a factor that could be associated with decreased sensitivity in negative urinary antigen (UAT) tests in CAP caused by pneumococcal. Prothrombin was 50% higher in the UAT-negative patients than in the UAT-positive patients [59]. We chose to include prothrombin in the diagnostic model to explore its significance in or rule out CAP,

			furthermore, the marker is routinely measured in acutely admitted patients.
	Bilirubin	Bilirubin µmol/L, median (IQR) Binary (Yes/no) Cut-off: <5 or >25 Yes= outside of the cut-off No= within the cut-off	Bilirubin levels were lower in patients with influenza A (H1N1) compared to non-H1N1 pneumonia (p= 0.02) [58]. This marker could add value to a prediction model.
	Glucose	Glucose mmol/L, median (IQR) Binary (Yes/no) Cut-off: > 11.00 Yes= >11.00 No= ≤ 11.00	Patients with CAP frequently present with admission hyperglycemia and have poorer outcomes [60, 61]. Therefore, glucose is included as a potential predictor.
	C- reactive protein (CRP)	C-Reactive Protein, median (IQR) Binary (Yes/no) The cut-off of CRP in our institution is < 5 mg/L at the ED. However, the literature suggests optional cut-offs. Based on the literature and the range of the results from the CRP as continuous variable, we defined the following categories: 1= <20mg/L 2= 20-100 mg/L 3= >100 mg/L	The diagnostic accuracy of CRP in differentiating between bacterial and viral infections of the lower respiratory tract is questionable [62]. However, CRP at different cut-offs increased the performance of prediction models for CAP. It included a cut-off of >20 [20], >30 [63], 50 [23] ≥ 98 [46], and a meta-analysis investigated all three cut-offs of 20, 50, and 100 [64]. CRP levels were found higher when CAP was detected both by a chest x-ray and a chest tomography [52].
Clinical assessment	Stethoscope findings	Binary (Yes/no) Yes for any abnormal stethoscope findings such as crackles and rhonchi.	Several studies investigated associations between abnormal stethoscope findings and the probability of the presence of CAP. They increased the likelihood of CAP [21, 65] and crackles on auscultation had a twofold increase in the prediction of pneumonia [19].
	Abdominal pain on palpation	Binary (Yes/no)	The rationale for including abdominal pain in the clinical assessment was that the literature reported that 20% of symptoms reported by patients with CAP were gastrointestinal symptoms [26].
	Body mass index (BMI).	The BMI was calculated including the high and weight of the patients. The BMI classification was based on "The Centers for diseases control and	The literature reported the association of several nutritional factors related to CAP and including malnutrition [1, 18], being underweight [8, 17], and BMI was directly associated with an increased risk of CAP among women [10].

		prevention" [66] and defined with the following categories: 1= Underweight, BMI < 18.5 2= Healthy weight, BMI from 18.5 to <25 3= Overweight, BMI from 25.0 to <30 4= Obesity, BMI from ≥ 30.0	

Table S2: Characteristics of CAP in the population of patients admitted with an infection (n=954). The values presented of data as continuous, dichotomous or categorical were tested in the model during explorative analysis to identify the best model performance.

Characteristics	Total, n	CAP, n	Not CAP, n	Missings n (%)	OR (95% CI)	p-value
Total of patients	954 (100)	265 (27.8)	689 (72.2)	0 (0.0)		
DEMOGRAPHIC DATA						
Age, median (IQR)	73.0, (59.0; 81.0)	75.0, (63.5;2.0)	73.0, (57.0;80.0)	0 (0.0)	1.01 (1.005-1.02)	<0.001
Age ≥75 years	440 (46.1)	133 (50.2)	307 (44.6)	0 (0.0)	1.25 (0.94-1.66)	0.118
Gender male	513 (53.8)	137 (51.7)	376 (54.6)	0 (0.0)	0.89 (0.67-1.18)	0.425
Marital status, Living alone	618 (66.0)	166 (63.8)	452 (66.9)	18 (1.9)	0.87 (0.64-1.18)	0.382
Nursing home resident	66 (7.0)	26 (9.9)	40 (5.9)	13 (1.4)	1.75 (1.05-2.94)	0.317
Occupation				21 (2.2)		
Others	67 (7.2)	17 (6.5)	50 (7.4)		1 (reference)	
Working	202 (21.7)	44 (16.9)	158 (23.5)		0.81 (0.43-1.55)	0.543
Retired	664 (71.2)	200 (76.6)	464 (69.0)		1.26 (0.71-2.25)	0.418
LIFESTYLE FACTORS						
Smoking status				33 (3.5)		
No	323 (35.1)	66 (26.0)	257 (38.5)		1 (reference)	
Current smoker	179 (19.4)	54 (21.3)	125 (18.7)		1.68 (1.10-2.55)	0.015
Previous smoker	419 (45.5)	134 (52.8)	285 (42.7)		1.83 (1.30-2.57)	<0.001
Alcohol status				35 (3.7)		

No alcohol	356 (38.7)	99 (39.1)	257 (38.6)		1 (reference)	
1-7 doses	385 (41.9)	105 (41.5)	280 (42.0)		0.97 (0.70-1.34)	0.870
8-14 doses	105 (11.4)	31 (12.3)	74 (11.1)		1.08 (0.67-1.75)	0.732
> 14 doses	73 (7.9)	18 (7.1)	55 (8.3)		0.84 (0.47-1.51)	0.582
Physically activity				52 (5.4)		
Not physical active	263 (29.2)	74 (29.8)	189 (28.9)		1 (reference)	
Physical activity < 2,5 hr/week	231 (25.6)	64 (25.8)	167 (25.5)		0.97 (0.66-1.45)	0.915
Physical activity ≥ 2,5 hr/week	408 (45.2)	110 (44.4)	298 (45.6)		0.94 (0.66-1.33)	0.735
Body Mass Index, median (IQR)	26.5 (23.2; 30.8)	26.2 (22.9; 29.5)	26.7 (23.3; 31.2)	249 (26.1)	0.97 (0.94-0.99)	0.031
Body Mass Index†				249 (26.1)		
Healthy weight	246 (34.9)	74 (36.1)	172 (34.4)		1 (reference)	
Obese	193 (27.4)	45 (22.0)	148 (29.6)		0.70 (0.45-1.08)	0.114
Overweight	239 (33.9)	74 (36.1)	165 (33.0)		1.04 (0.70-1.53)	0.833
Underweight	27 (3.8)	12 (5.9)	15 (3.0)		1.85 (0.83-4.16)	0.132
ADL dependence*	260 (28.0)	81 (31.2)	179 (26.8)	25 (2.6)	1.23 (0.90-1.69)	0.180
SYMPTOMS						
Feeling unwell	559 (61.2)	173 (67.8)	386 (58.7)	41 (4.3)	1.48 (1.09-2.01)	0.010
Feeling tired	657 (72.6)	190 (75.4)	467 (71.5)	49 (5.1)	1.22 (0.87-1.70)	0.241
Headache	351 (38.3)	99 (38.8)	252 (38.1)	37 (3.9)	1.03 (0.76-1.38)	0.832
Dizziness	346 (37.7)	96 (37.6)	250 (37.8)	37 (3.98)	0.99 (0.73-1.34)	0.973
Confusion	207 (22.6)	58 (22.7)	149 (22.5)	37 (3.89)	1.01 (0.71-1.43)	0.938
Dyspnea	379 (41.4)	171 (67.3)	208 (31.5)	39 (4.1)	4.48 (3.29-6.11)	<0.001
Cough	358 (39.1)	173 (68.1)	185 (28.0)	39 (4.1)	5.49 (4.01-7.52)	<0.001
Expectoration	279 (30.5)	140 (55.1)	139 (21.0)	39 (4.1)	4.61 (3.38-6.28)	<0.001
Sore throat	104 (11.4)	39 (15.4)	65 (9.8)	39 (4.1)	1.66 (1.08-2.54)	0.019
Cold (common cold)	95 (10.4)	45 (17.7)	50 (7.6)	39 (4.1)	2.63 (1.70-4.05)	<0.001
Fever feling at home	612 (64.2)	169 (63.8)	443 (64.3	0 (0.0)	0.97 (0.72-1.31)	0.880
Chest pain	168 (18.4)	71 (28.1)	97 (14.7)	40 (4.2)	2.26 (1.60-3.21)	<0.001
Oedema	79 (8.6)	10 (4.0)	69 (10.4)	39 (4.1)	0.35 (1,17-0.69)	0.002
Nausea	304 (33.2)	76 (30.0)	228 (34.4)	38 (3.9)	0.81 (0.59-1.112)	0.211

Vomiting	190 (20.7)	40 (15.8)	150 (22.6)	38 (3.9)	0.64 (0.43-0.94)	0.023
Loss of appetite	524 (57.2)	149 (58.9)	375 (56.6)	38 (3.9)	1.00 (0.82-1.47)	0.523
Gastrointestinal pain	193 (21.1)	40 (15.8)	153 (23.1)	38 (3.9)	0.62 (0.42-0.91)	0.016
Diarrhoea	134 (14.6)	29 (11.5)	105 (15.8)	38 (3.9)	0.68 (0.44-1.06)	0.095
Muscular pain	344 (37.8)	79 (31.3)	265 (40.3)	44 (4.6)	0.67 (0.49-0.92)	0.013
Back pain	132 (14.5)	33 (13.1)	99 (15.0)	44 (4.6)	0.85 (0.55-1.29)	0.455
CLINICAL ASSESSMENT						
Positive stethoscope findings	329 (36.5)	168 (65.4)	161 (25.0)	52 (5.4)	5.67 (4.15-7.75)	<0.001
Abdominal pain by palpation	192 (22.1)	37 (15.0)	155 (25.0)	86 (9.0)	0.52 0.35-0.78)	0.002
COMORBIDITIES						
Dementia	32 (3.4)	9 (3.4)	23 (3.3)	0 (0.0)	1.01 (0.46-2.22)	0.964
Neurological diseases	172 (18.0)	53 (20.0)	119 (17.3)	0 (0.0)	1.19 (0.83-1.71)	0.326
Respiratory diseases	269 (28.2)	105 (39.6)	164 (23.8)	0 (0.0)	2.10 (1.55-2.84)	<0.001
Endocrinological diseases	296 (31.0)	80 (30.2)	216 (31.3)	0 (0.0)	0.94 (0.69-1.28)	0.728
Nephrological diseases	252 (26.4)	60 (22.6)	192 (27.9)	0 (0.0)	0.75 (0.54-1.05)	0.101
Cardiovascular diseases	390 (40.9)	116 (43.8)	274 (39.8)	0 (0.0)	1.17 (0.88-1.57)	0.259
Gastrointestinal diseases	100 (10.5)	23 (8.7)	77 (11.2)	0 (0.0)	0.75 (0.46-1.23)	0.260
Rheumatological diseases	118 (12.4)	27 (10.2)	91 (13.2)	0 (0.0)	0.74 (0.47-1.17)	0.205
Cancer diseases	85 (8.9)	26 (9.8)	59 (8.6)	0 (0.0)	1.16 (0.71-1.88)	0.544
Prior pneumonia				100 (10.5)		
No	410 (48.0)	79 (33.3)	331 (53.6)		1 (reference)	
Yes, one time	180 (21.1)	50 (21.1)	130 (21.1)		1.61 (1.07-2.42)	0.022
Yes, more than one time	264 (30.9)	108 (45.6)	156 (25.3)		2.90 (2.05-4.10)	<0.001
SEVERITY ASSESSMENT						
CURB65 ≥3 **	122 (13.0)	29 (11.3)	93 (13.7)	16 (1.7)	0.80 (0.51-1.25)	0.336
Triage***				59 (6.2)		
Green/Blue	183 (20.4)	37 (14.8)	146 (22.6)		1 (reference)	
Yellow	479 (53.5)	126 (50.4)	353 (54.7)		1.40 (0.93-2.13)	0.105
Red/Orange	233 (26.0)	87 (34.8)	146 (22.6)		2.35 (1.50-3.67)	<0.001
VITAL PARAMETERS						

Respiratory rate, median(IQR)	18.0 (16.0; 22.0)	20.0 (18.0; 24.0)	18.0 (16.0; 20.0)	5 (0.5)	1.10 (1.07-1.13)	<0.001
Respiratory rate >20/min	285 (30.0)	124 (47.0)	161 (23.5)	5 (0.5)	2.88 (2.13-3.88)	<0.001
Oxygen saturation % n/min, median (IQR)	96.0 (94.0; 98.0)	95.0 (93.0; 97.0)	97.0 (95.0; 98.0)	4 (0.4)	0.84 (0.80-0.88)	<0.001
Oxygen saturation < 96 %	393 (41.4)	162 (61.1)	231 (33.7)	4 (0.4)	3.09 (2.30-4.14)	<0.001
Heart rate/min, mean (sd)	90.1 (18.3)	93.2 (18.9)	88.9 (18.0)	1 (0.1)	1.01 (1.005-1.02)	0.001
Heart rate <51 or >90/min	460 (48.3)	148 (55.8)	312 (45.3)	1 (0.1)	1.52 (1.14-2.02)	0.003
Systolic blood pressure mmHg, mean (sd)	132.8 (22.5)	134.2(21.0)	132.2 (23.1)	3 (0.3)	1.003 (0.99-1.01	0.215
Systolic blood pressure <111 or >219 mmHg	156 (16.4)	38 (14.4)	118 (17.2)	3 (0.3)	0.81 (0.54-1.21)	0.314
Diastolic blood pressure mmHg, mean (sd)	74.8 (15.3)	74.2 (13.6)	75.0 (15.8)	3 (0.3)	0.99 (0.98-1.006)	0.483
Diastolic blood pressure ≤60 mmHg	163 (17.1)	40 (15.2)	123 (17.9)	3 (0.3)	0.82 (0.55-1.21)	0.329
Temperature, mean (SD)	37.5 (1.0)	37.6 (1.0)	37.4 (0.9)	5 (0.5)	1.22 (1.05-1.40)	0.006
Fever > 38ºC	233 (24.6)	77 (29.3)	156 (22.7)	5 (0.5)	1.40 (1.02-1.93)	0.036
Glascow coma scale <15	31 (3.3)	12 (4.6)	19 (2.8)	5 (0.5)	0.59 (0.28-1.24)	0.168
BLOOD TESTS						
Haematocrit, median (IQR)	38.0 (35.0; 42.0)	38.0 (35.0; 42.0)	39.0 (35.0; 42.0)	260 (27.2)	0.98 (0.95-1.01)	0.465
Haematocrit	268 (38.6)	85 (38.6)	183 (38.6)	260 (27.2)	1.001 (0.72-1.39)	0.994
Haemoglobin mmol/L, median (IQR)	8.0 (7.2; 8.7)	7.9 (7.2; 8.6)	8.0 (7.3; 8.8)	0 (0.0)	0.90 (0.80-1.02)	0.127
Haemoglobin mmol/L	402 (42.1)	118 (44.5)	284 (41.2)	0 (0.0)	1.14 (0.86-1.52)	0.354
Leukocytes 10E9/L, median (IQR)	11.1 (8.3; 14.8)	12.2 (9.5; 15.8)	10.7 (8.0; 14.2)	0 (0.0)	1.05 (1.02-1.07)	<0.001
Leukocytes 10E9/L	670 (70.2)	214 (80.8)	456 (66.2)	0 (0.0)	2.14 (1.52-3.02)	<0.001
Platelets 10E9/L, median (IQR)	240.0 (189.0; 307.8)	260.5 (211;330.8)	232.0 (182.3; 296.0)	10 (1.0)	1.002 (1.001-1.004)	<0.001
Platelets 10E9/L	201 (21.3)	63 (23.9)	138 (20.3)	10 (1.0)	1.23 (0.87-1.72)	0.229
Neutrophilocytes 10E9/L, median (IQR)	8.4 (6.0; 12.2)	9.7 (7.2; 13.0)	8.0 (5.6; 11.6)	10 (1.0)	1.06 (1.03-1.09)	<0.001
Neutrophilocytes 10E9/L	549 (58.2)	187 (71.1)	362 (53.2)	10 (1.0)	2.16 (1.59-2.94)	<0.001
Lymphocytes† 10E9/L, median (IQR)	1.1 (0.7; 1.6)	0.9 (0.6; 1.5)	1.2 (0.8; 1.8)	633 (66.3)	0.98 (0.85-1.12)	0.797
Lymphocytes† 10E9/L	145 (45.2)	53 (55.2)	92 (40.9)	633 (66.3)	1.78 (1.10-2.88)	0.018

Albumin g/L, median (IQR)	39.0 (36.0; 42.0)	39.0 (35.0; 41.0)	39.0 (36.0; 42.0)	7 (0.7)	0.96 (0.93-0.99)	0.029
Albumin g/L	160 (16.9)	39 (14.9)	121 (17.6)	7 (0.7)	0.82 (0.55-1.21)	0.323
Creatinine µmol/L, median (IQR)	84.0 (67.0; 113.0)	81.0 (64; 108.0)	86.0 (67.5; 114.0)	0 (0.0)	0.996 (0.993-0.998)	0.003
Creatinine µmol/L	374 (39.2)	106 (40.0)	268 (38.9)	0 (0.0)	1.04 (0.78-1.39)	0.754
Blood urea nitrogen mmol/L, median (IQR)	6.2 (4.4; 8.9)	6.2 (4.5; 8.6)	6.2 (4.4; 9.1)	9 (0.9)	0.99 (0.96-1.02)	0.657
Blood urea nitrogen mmol/L	377 (39.9)	99 (38.1)	278 (40.6)	9 (0.9)	0.90 (0.67-1.20)	0.482
Natrium mmol/L, median (IQR)	137.0 (134.0; 139.0)	137.0 (134; 139)	137.0 (134.0; 139.0)	0 (0.0)	0.98 (0.95-1.01)	0.394
Natrium mmol/L	432 (45.3)	128 (48.3)	304 (44.1)	0 (0.0)	1.18 (0.89-1.57)	0.245
Prothrombin, median (IQR)	1.1 (1.0; 1.2)	1.1 (1.0; 1.2)	1.1 (1.0; 1.2)	3 (0.3)	1.18 (0.89-1.58)	0.231
Prothrombin	234 (24.6)	65 (24.5)	169 (24.6)	3 (0.3)	0.99 (0.71-1.38)	0.972
Bilirubin µmol/L, median (IQR)	9.0 (6.0; 13.0)	9.0 (6.0; 12.0)	9.0 (6.0; 14.0)	11 (1.1)	0.97 (0.95-0.99)	0.254
Bilirubin µmol/L	152 (16.1)	38 (14.4)	114 (16.8)	11 (1.1)	0.83 (0.55-1.24)	0.369
Glucose mmol/L, median (IQR)	6.7 (5.9; 7.9)	6.9 (6.2; 8.1)	6.6 (5.8; 7.8)	9 (0.9)	1.04 (0.99-1.10)	0.052
Glucose mmol/L	51 (5.4)	19 (7.3)	32 (4.7)	9 (0.9)	1.59 (0.88-2.85)	0.120
C-Reactive Protein mg/L, median (IQR)	95.5 (30.0; 179.3)	125.0 (57; 203.5)	82.0 (19.0; 172.0)	0 (0.0)	1.003 (1.001-1.004)	<0.001
C-Reactive Protein mg/L				0 (0.0)		
Low <20mg/L	196 (20.5)	21 (7.9)	175 (25.4)		1 (reference)	
Moderate 21-99 mg/L	291 (30.5)	86 (32.5)	205 (29.8)		3.49 (2.08-5.86)	<0.001
High >=100	467 (49.0)	158 (59.6)	309 (44.8)		4.26 (2.60-6.96)	<0.001
VACCINE AND MEDICAMEN-TATIONS						
SARS-CoV-2 †	756 (79.2)	222 (83.8)	534 (77.5)	0 (0.0)	1.49 (1.03-2.17)	0.033
Pneumococcal	530 (55.6)	160 (60.4)	370 (53.7)	0 (0.0)	1.31 (0.98-1.75)	0.063
Influenza	635 (66.6)	191 (72.1)	444 (64.4)	0 (0.0)	1.42 (1.04-1.94)	0.025
Analgesics	404 (42.3)	115 (43.4)	289 (41.9)	0 (0.0)	1.06 (0.79-1.41)	0.684
Polypharmacy****	544 (57.0)	163 (61.5)	381 (55.3)	0 (0.0)	1.29 (0.96-1.72)	0.082

Values are numbers (percentages) unless otherwise specified. *ADL dependence: If the patient had one or more dependencies regarding bathing, dressing, toileting, transfer, continence, and feeding. ** CURB65: confusion, uraemia, respiratory rate, blood pressure, age > 65 years. ***Triage: Danish emergency process triage [40] ****Polypharmacy: regular consumption of at least five medications † variables not included in the multivariate model

Table S3: Characteristics of the 954 patients with suspected infection enrolled in the study. It presents the 70 predictors included in the multivariate analysis and randomization of the training set and validation set.

Characteristics	Total, n	Training set, n	Validation set, n	Missings n (%)	p-value
Total of patients	954 (100)	766 (80.3)	188 (19.7)	0 (0.0)	
DEMOGRAPHIC DATA					
Age, median (IQR)	73.0 (59.0;81.0)	75.0 (63.5; 82.0)	74.0 (60.0; 82.0)	0 (0.0)	0.54
Age ≥75 years	440 (46.1)	348 (45.4)	92 (48.9)	0 (0.0)	0.39
Gender male	513 (53.8)	408 (53.3)	105 (55.9)	0 (0.0)	0.52
Marital status, Living alone	618 (66.0)	488 (65.0)	130 (70.3)	18 (1.9)	0.17
Nursing home resident	66 (7.0)	55 (7.3)	11 (5.9)	13 (1.4)	0.53
Occupation				21 (2.2)	0.62
Others	67 (7.2)	57 (7.6)	10 (5.5)		
Working	202 (21.7)	162 (21.6)	40 (22.0)		
Retired	664 (71.2)	532 (70.8)	132 (72.5)		
LIFESTYLE FACTORS					
Smoking status				33 (3.5)	0.76
No	323 (35.1)	256 (34.5)	67 (37.4)		
Current smoker	179 (19.4)	145 (19.5)	34 (19.0)		
Previous smoker	419 (45.5)	341 (46.0)	78 (43.6)		
Alcohol status				35 (3.7)	0.60
No alcohol	356 (38.7)	283 (38.2)	73 (40.8)		
1-7 doses	385 (41.9)	315 (42.6)	70 (39.1)		
8-14 doses	105 (11.4)	81 (10.9)	24 (13.4)		
> 14 doses	73 (7.9)	61 (8.2)	12 (6.7)		
Physically activity				52 (5.4)	0.76
Not physical active	263 (29.2)	214 (29.4)	49 (28.2)		
Physical activity < 2,5 hr/week	231 (25.6)	189 (26.0)	42 (24.1)		
Physical activity ≥ 2,5 hr/week	408 (45.2)	325 (44.6)	83 (47.7)		
Body Mass Index†				249 (26.1)	0.74
Healthy weight	246 (34.9)	202 (35.8)	44 (31.2)		
Obese	193 (27.4)	154 (27.3)	39 (27.7)		
Overweight	239 (33.9)	187 (33.2)	52 (36.9)		
Underweight	27 (3.8)	21 (3.7)	6 (4.3)		
ADL dependence*	260 (28.0)	203 (27.1)	57 (31.7)	25 (2.6)	0.22
SYMPTOMS					

Malaise	559 (61.2)	458 (62.0)	101 (58.0)	41 (4.3)	0.34
Feeling tired	657 (72.6)	540 (74.0)	117 (66.9)	49 (5.1)	0.06
Headache	351 (38.3)	287 (38.8)	64 (36.0)	37 (3.9)	0.48
Dizziness	346 (37.7)	287 (38.8)	59 (33.1)	37 (3.98)	0.16
Confusion	207 (22.6)	164 (22.2)	43 (24.2)	37 (3.89)	0.57
Dyspnea	379 (41.4)	309 (42.0)	70 (39.1)	39 (4.1)	0.48
Cough	358 (39.1)	294 (39.9)	64 (35.8)	39 (4.1)	0.30
Fever feeling at home	612 (64.2)	464 (64.5)	118 (62.8)	0 (0.0)	0.66
Expectoration	279 (30.5)	224 (30.4)	55 (30.7)	39 (4.1)	0.94
Sore throat	104 (11.4)	86 (11.7)	18 (10.1)	39 (4.1)	0.54
Cold (common cold)	95 (10.4)	81 (11.0)	14 (7.8)	39 (4.1)	0.21
Chest pain	168 (18.4)	134 (18.2)	34 (19.0)	40 (4.2)	0.81
Oedema	79 (8.6)	61 (8.3)	18 (10.1)	39 (4.1)	0.45
Nausea	304 (33.2)	247 (33.4)	57 (32.2)	38 (3.9)	0.76
Vomiting	190 (20.7)	154 (20.8)	36 (20.3)	38 (3.9)	0.88
Loss of appetite	524 (57.2)	424 (57.4)	100 (56.5)	38 (3.9)	0.83
Gastrointestinal pain	193 (21.1)	145 (19.6)	48 (27.1)	38 (3.9)	0.03
Diarrhoea	134 (14.6)	107 (14.5)	27 (15.3)	38 (3.9)	0.79
Muscular pain	344 (37.8)	289 (39.5)	55 (30.9)	44 (4.6)	0.03
Back pain	132 (14.5)	110 (15.0)	22 (12.4)	44 (4.6)	0.36
CLINICAL ASSESSMENT					
Positive stethoscope findings	329 (36.5)	263 (36.5)	66 (36.5)	52 (5.4)	1.00
Abdominal pain by palpation	192 (22.1)	151 (21.7)	41 (23.7)	86 (9.0)	0.58
COMORBIDITIES					
Dementia	23 (3.0)	9 (4.8)	23 (3.3)	0 (0.0)	0.22
Neurological diseases	137 (17.9)	35 (18.6)	119 (17.3)	0 (0.0)	0.82
Pulmonary diseases	212 (27.7)	57 (30.3)	164 (23.8)	0 (0.0)	0.47
Endocrinological diseases	239 (31.2)	57 (30.3)	216 (31.3)	0 (0.0)	0.81
Nephrological diseases	200 (26.1)	52 (27.7)	192 (27.9)	0 (0.0)	0.67
Cardiovascular diseases	303 (39.6)	87 (46.3)	274 (39.8)	0 (0.0)	0.09
Gastrointestinal diseases	81 (10.6)	19 (10.1)	77 (11.2)	0 (0.0)	0.85
Rheumatological diseases	93 (12.1)	25 (13.3)	91 (13.2)	0 (0.0)	0.67
Cancer diseases	66 (8.6)	19 (10.1)	59 (8.6)	0 (0.0)	0.52
Prior pneumonia				100 (10.5)	0.05
No	343 (50.1)	67 (39.6)	331 (53.6)		
Yes, one time	139 (20.3)	41 (24.3)	130 (21.1)		

Yes, more than one time	203 (29.6)	61 (36.1)	156 (25.3)		
SEVERITY ASSESSMENT					
CURB65 ≥3 **	103 (13.6)	19 (10.4)	93 (13.7)	16 (1.7)	0.25
Triage***				59 (6.2)	0.53
Green/Blue	185 (25.6)	48 (27.9)	146 (22.6)		
Yellow	385 (53.3)	94 (54.7)	353 (54.7)		
Red/Orange	153 (21.2)	30 (17.4)	146 (22.6)		
VITAL PARAMETERS					
Respiratory rate >20/min	285 (30.0)	235 (30.8)	50 (26.7)	5 (0.5)	0.27
Oxygen saturation < 96 %	393 (41.4)	324 (42.5)	69 (36.7)	4 (0.4)	0.15
Heart rate <51 or >90/min	460 (48.3)	377 (49.3)	83 (44.1)	1 (0.1)	0.21
Systolic blood pressure <111 or >219 mmHg	156 (16.4)	125 (16.4)	31 (16.6)	3 (0.3)	0.94
Diastolic blood pressure ≤60 mmHg	163 (17.1)	131 (17.1)	32 (17.1)	3 (0.3)	0.99
Fever > 38ºC	233 (24.6)	190 (24.9)	43 (23.1)	5 (0.5)	0.61
Glascow coma scale <15	31 (3.3)	23 (3.0)	8 (4.3)	5 (0.5)	0.39
BLOOD TESTS					
Haematocrit	268 (38.6)	218 (39.2)	50 (36.2)	260 (27.2)	0.52
Haemoglobin mmol/L	402 (42.1)	329 (43.0)	73 (38.8)	0 (0.0)	0.31
Leukocytes 10E9/L	670 (70.2)	548 (71.5)	122 (64.9)	0 (0.0)	0.07
Platelets 10E9/L	201 (21.3)	168 (22.2)	33 (17.6)	10 (1.0)	0.17
Neutrophilocytes 10E9/L	549 (58.2)	454 (59.9)	95 (51.1)	10 (1.0)	0.03
Albumin g/L	160 (16.9)	130 (17.1)	30 (16.1)	7 (0.7)	0.76
Creatinine µmol/L	374 (39.2)	303 (39.6)	71 (37.8)	0 (0.0)	0.65
Blood urea nitrogen mmol/L	377 (39.9)	308 (40.5)	69 (37.5)	9 (0.9)	0.46
Natrium mmol/L	432 (45.3)	362 (47.3)	70 (37.2)	0 (0.0)	0.01
Prothrombin	234 (24.6)	186 (24.3)	48 (25.7)	3 (0.3)	0.71
Bilirubin µmol/L	152 (16.1)	119 (15.7)	33 (17.8)	11 (1.1)	0.48
Glucose mmol/L	51 (5.4)	42 (5.5)	9 (4.8)	9 (0.9)	0.71
C-Reactive Protein mg/L				0 (0.0)	0.07
<20 mg/L	196 (20.5)	151 (19.7)	45 (23.9)		
21-99 mg/L	291 (30.5)	226 (29.5)	65 (34.6)		
≥ 100 mg/L	467 (49.0)	389 (50.8)	78 (41.5)		
VACCINE AND MEDICAMENTATIONS					
Pneumococcal	530 (55.6)	414 (54.0)	116 (61.7)	0 (0.0)	0.06
Influenza	635 (66.6)	512 (66.8)	123 (65.4)	0 (0.0)	0.71

Analgesics	404 (42.3)	336 (43.9)	68 (36.2)	0 (0.0)	0.06
Polypharmacy****	544 (57.0)	443 (57.8)	101 (53.7)	0 (0.0)	0.31

Values are numbers (percentages) unless otherwise specified. *ADL dependence: If the patient had one or more dependencies regarding bathing, dressing, toileting, transfer, continence, and feeding. ** CURB65: confusion, uraemia, respiratory rate, blood pressure, age > 65 years. ***Triage: Danish emergency process triage [40] ****Polypharmacy: regular consumption of at least five medications

Figure S4: Performance of the prediction model presented with the area receiver operating characteristic curve

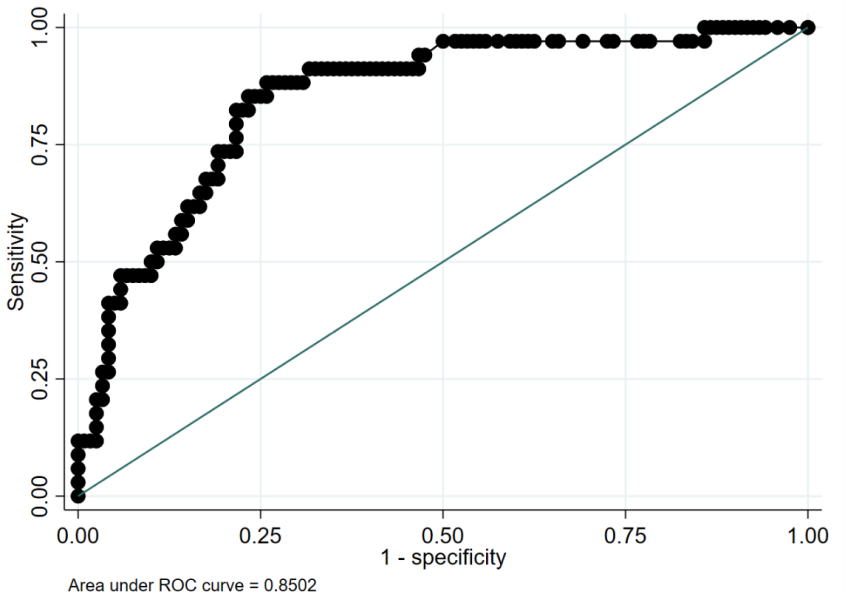
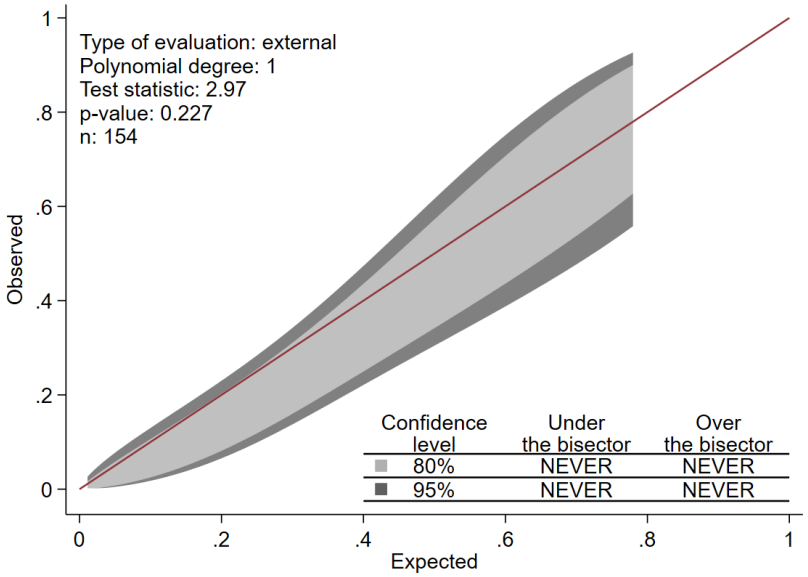


Figure S5: The calibration of the model after recalibration



Formula S6: Based on a lambda result of λ=0.0402856 and a probability threshold of 0.35, the LASSO calculation with characteristics predictive of CAP as follows:

$$
\begin{aligned}
CAP-score = {} & 0.07 \cdot 1_{Unwell=yes} + 0.35 \cdot 1_{Dyspnea=yes} + 0.36 \\
& \cdot 1_{Expectoration=yes} + 0.39 \cdot 1_{Cough=yes} + 0.34 \cdot 1_{Cold=yes} \\
& + 0.14 \cdot 1_{Respiratory\ rate\ >20/min=yes} + 0.24 \\
& \cdot 1_{Oxygen\ saturation<96\%=yes} + 0.20 \cdot 1_{Chest\ pain=yes} + 0.56 \\
& \cdot 1_{Stethoscope=yes} - 0.12 \cdot 1_{Previous\ CAP=no} + 0.003 \\
& \cdot 1_{Leucocytes<3.5\ or\ >8.8\ 10E9\ /L=yes} + 0.08 \\
& \cdot 1_{Neutrophilocytes>7.5\ 10E9\ /L=yes} - 0.64 \cdot 1_{CRP<20mg\ /L=yes} \\
& + 0.53 \cdot 1_{Cough=yes} \cdot 1_{age\geq 75} - 0.05.\, 1_{Edema=yes} \cdot 1_{age\geq 75} \\
& + 0.88 \cdot 1_{Glucose>11\ mmol\ /L=yes} \cdot 1_{age\geq 75} + 0.0402856 \\
& \cdot (0.07 + 0.35 + 0.36 + 0.39 + 0.015 + 0.34 + 0.14 + 0.24 \\
& + 0.20 + 0.56 + 0.12 + 0.003 + 0.08 + 0.64 + 0.53 + 0.05 \\
& + 0.88) - 1.66192 - \log\left(\frac{0.35}{0.65}\right)
\end{aligned}
$$

For best calibration, 0.07 must be subtracted from the score if the score is between 0.08 and 0.47.

Formula S7: A cutoff value greater than 0 indicates the diagnosis CAP according to our model and can be calculated using the following formula:

$$
\begin{aligned}
CAP-score = {} & 0.07 \cdot 1_{Unwell=yes} + 0.35 \cdot 1_{Dyspnea=yes} + 0.36 \\
& \cdot 1_{Expectoration=yes} + 0.39 \cdot 1_{Cough=yes} + 0.34 \cdot 1_{Cold=yes} \\
& + 0.14 \cdot 1_{Respiratory\ rate\ >20/min=yes} + 0.24 \\
& \cdot 1_{Oxygen\ saturation<96\%=yes} + 0.20 \cdot 1_{Chest\ pain=yes} + 0.56 \\
& \cdot 1_{Stethoscope=yes} - 0.12 \cdot 1_{Previous\ CAP=no} + 0.003 \\
& \cdot 1_{Leucocytes<3.5\ or\ >8.8\ 10E9\ /L=yes} + 0.08 \\
& \cdot 1_{Neutrophilocytes>7.5\ 10E9\ /L=yes} - 0.64 \cdot 1_{CRP<20mg\ /L=yes} \\
& + 0.53 \cdot 1_{Cough=yes} \cdot 1_{age\geq 75} - 0.05.\, 1_{Edema=yes} \cdot 1_{age\geq 75} \\
& + 0.88 \cdot 1_{Glucose>11\ mmol\ /L=yes} \cdot 1_{age\geq 75} - 0.842742
\end{aligned}
$$

For best calibration, 0.07 must be subtracted from the score if the score is between 0.08 and 0.47.

Model specification

Besides the high percentage of missings from lymphocytes (66.3%), lymphocytes contributed to a significantly decreased model performance below 80% and a narrower calibration belt ($p<0.001$), furthermore lymphocytes were missing for 66.3% of the patients. SARS-CoV-2 vaccine was not included in the final model as the vaccine was related to a specific pandemic and did not change any final predictors or values. The inclusion of the BMI had better prediction performance AUC: 0.86 (CI: 0.79-0.93) and yielded more predictors especially related to lifestyle. The predictors that differed from the final model were: Alcohol (8-14 doses/week) 0.01792, level of physical activity under 2,5 hours/week yielded 0.01067, and obesity appeared with a coefficient of -0.93861. In addition, a symptom of diarrhea (-0.17572), muscular pain (-0.00225), gastrointestinal symptoms (-0.807885) sore throat (0.074709 for patients $\geq$ 75 years old) and the presence of nephrological diseases (-0.18776 for patients $\geq$ 75 years old) were predictors of CAP in the model constructed including BMI. From a clinical perspective, we chose to exclude the BMI as the final model would be more useful in an acute setting where reliable information about BMI is not always available. From a statistical perspective, BMI had almost 27% of missings, which would be classified as MAR and possibly selected from the population.

References

1. Almirall, J., et al., *Risk Factors for Community-Acquired Pneumonia in Adults: A Systematic Review of Observational Studies.* Respiration, 2017. 94(3): p. 299-311.
2. Riquelme, R., et al., *Community-acquired pneumonia in the elderly. Clinical and nutritional aspects.* Am J Respir Crit Care Med, 1997. 156(6): p. 1908-14.
3. Janssens, J.P., *Pneumonia in the elderly (geriatric) population.* Curr Opin Pulm Med, 2005. 11(3): p. 226-30.
4. Cillóniz, C., et al., *Community-acquired pneumonia in critically ill very old patients: a growing problem.* Eur Respir Rev, 2020. 29(155).
5. Metlay, J.P., et al., *Influence of age on symptoms at presentation in patients with community-acquired pneumonia.* Arch Intern Med, 1997. 157(13): p. 1453-9.
6. Laporte, L., et al., *Ten-year trends in intensive care admissions for respiratory infections in the elderly.* Ann Intensive Care, 2018. 8(1): p. 84.
7. Ravioli, S., et al., *Age- and sex-related differences in community-acquired pneumonia at presentation to the emergency department: a retrospective cohort study.* Eur J Emerg Med, 2022. 29(5): p. 366-372.
8. Hammond, A., et al., *Predisposing factors to acquisition of acute respiratory tract infections in the community: a systematic review and meta-analysis.* BMC Infect Dis, 2021. 21(1): p. 1254.
9. Barbagelata, E., et al., *Gender differences in community-acquired pneumonia.* Minerva Med, 2020. 111(2): p. 153-165.
10. Baik, I., et al., *A prospective study of age and lifestyle factors in relation to community-acquired pneumonia in US men and women.* Arch Intern Med, 2000. 160(20): p. 3082-8.
11. Heath, G.W., et al., *Exercise and the incidence of upper respiratory tract infections.* Medicine and science in sports and exercise, 1991. 23(2): p. 152-157.
12. Kim, N.E., et al., *Clinical characteristics and outcomes among older nursing home residents hospitalized with pneumonia.* Arch Gerontol Geriatr, 2021. 95: p. 104394.
13. Nieman, D.C., et al., *Upper respiratory tract infection is reduced in physically fit and active adults.* British journal of sports medicine, 2011. 45(12): p. 987-992.

14. Calvillo–King, L., et al., *Impact of social factors on risk of readmission or mortality in pneumonia and heart failure: systematic review.* Journal of general internal medicine, 2013. 28(2): p. 269-282.
15. Clyde, W.A., Jr., *Clinical overview of typical Mycoplasma pneumoniae infections.* Clin Infect Dis, 1993. 17 Suppl 1: p. S32-6.
16. Gamble, J.M., et al., *Medication transitions and polypharmacy in older adults following acute care.* Ther Clin Risk Manag, 2014. 10: p. 189-96.
17. Torres, A., et al., *Risk factors for community-acquired pneumonia in adults in Europe: a literature review.* Thorax, 2013. 68(11): p. 1057-65.
18. Riquelme, R., et al., *Community-acquired pneumonia in the elderly: clinical and nutritional aspects.* Revista médica de Chile, 2008. 136(5): p. 587-593.
19. Moore, M., et al., *Predictors of pneumonia in lower respiratory tract infections: 3C prospective cough complication cohort study.* Eur Respir J, 2017. 50(5).
20. Ding, F., et al., *Development and validation of a simple tool composed of items on dyspnea, respiration rates, and C-reactive protein for pneumonia prediction among acute febrile respiratory illness patients in primary care settings.* BMC Med, 2022. 20(1): p. 360.
21. Nakanishi, M., et al., *Significance of the progression of respiratory symptoms for predicting community-acquired pneumonia in general practice.* Respirology, 2010. 15(6): p. 969-74.
22. Huijskens, E.G.W., et al., *The value of signs and symptoms in differentiating between bacterial, viral and mixed aetiology in patients with community-acquired pneumonia.* J Med Microbiol, 2014. 63(Pt 3): p. 441-452.
23. Loubet, P., et al., *Community-acquired pneumonia in the emergency department: an algorithm to facilitate diagnosis and guide chest CT scan indication.* Clin Microbiol Infect, 2020. 26(3): p. 382.e1-382.e7.
24. Beekman, R., et al., *Validating a clinical prediction score for Legionella-related community acquired pneumonia.* BMC Infect Dis, 2022. 22(1): p. 442.
25. den Engelsen, C., et al., *Infectious diseases and the use of antibiotics in outpatients at the emergency department of the University Hospital of León, Nicaragua.* Int J Infect Dis, 2009. 13(3): p. 349-54.

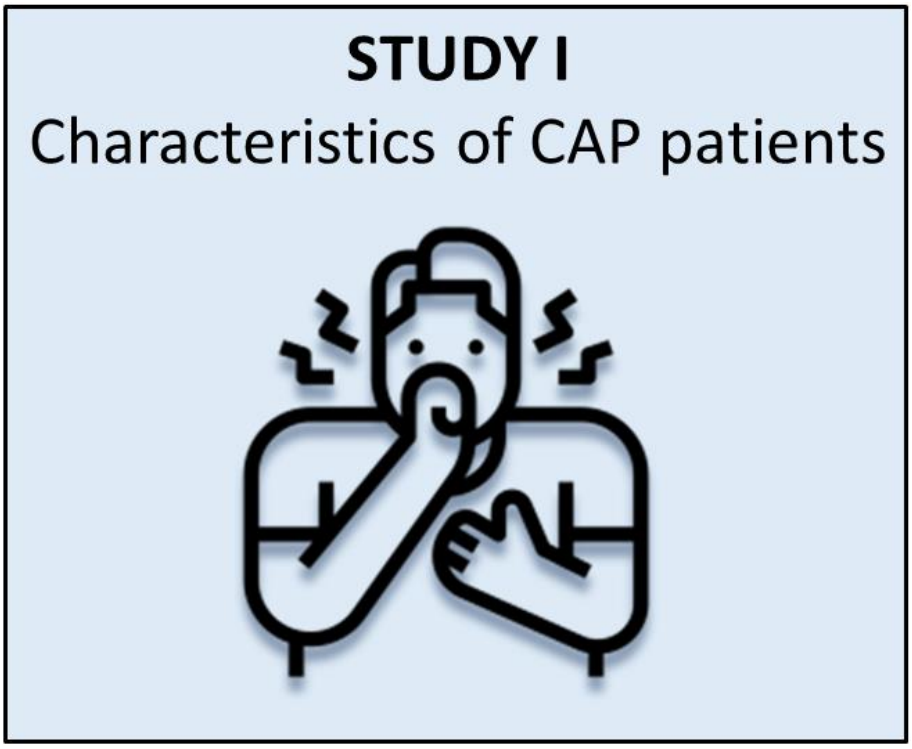
26. Mandell, L.A., *Community-acquired pneumonia: An overview.* Postgrad Med, 2015. 127(6): p. 607-15.
27. Takase, R., et al., *Clinical Manifestations of Patients with Influenza Differ by Age : A Prospective, Multi-centered Study in the Setouchi Marine Area.* Acta Med Okayama, 2021. 75(5): p. 567-574.
28. Akhtar, A., et al., *Respiratory-tract infections among geriatrics: prevalence and factors associated with the treatment outcomes.* Therapeutic advances in respiratory disease, 2021. 15: p. 1753466620971141.
29. Sundhedsstyrelsen. *Sundhedsstyrelsens udmeldinger om alkohol*. 2022 [cited 2022 December 06]; Available from: https://www.sst.dk/da/Viden/Forebyggelse/Alkohol/Alkoholforebyggelse/Sundhedsstyrelsens-udmeldinger-om-alkohol
30. (WHO), W.H.O. [cited 2022 December 6]; Available from: chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://apps.who.int/iris/bitstream/handle/10665/337001/9789240014886-eng.pdf
31. Shang, J., et al., *Risk factors for infection in home health care: Analysis of national Outcome and Assessment Information Set data.* Res Nurs Health, 2020. 43(4): p. 373-386.
32. Guidet, B., et al., *Caring for the critically ill patients over 80: a narrative review.* Ann Intensive Care, 2018. 8(1): p. 114.
33. Steffens, C., et al., *The Association Between Prescribed Opioid Receipt and Community-Acquired Pneumonia in Adults: a Systematic Review and Meta-analysis.* J Gen Intern Med, 2020. 35(11): p. 3315-3322.
34. Walters, J.A., et al., *Pneumococcal vaccines for preventing pneumonia in chronic obstructive pulmonary disease.* Cochrane Database Syst Rev, 2017. 1(1): p. Cd001390.
35. Kraicer-Melamed, H., S. O'Donnell, and C. Quach, *The effectiveness of pneumococcal polysaccharide vaccine 23 (PPV23) in the general population of 50 years of age and older: A systematic review and meta-analysis.* Vaccine, 2016. 34(13): p. 1540-1550.
36. Liang, C.Y., et al., *Effectiveness of influenza vaccination in the elderly: a population-based case-crossover study.* BMJ Open, 2022. 12(2): p. e050594.
37. Chalmers, J.D., et al., *Severity assessment tools for predicting mortality in hospitalised patients with community-acquired pneumonia. Systematic review and meta-analysis.* Thorax, 2010. 65(10): p. 878-83.

38. Lim, W.S., et al., *BTS guidelines for the management of community acquired pneumonia in adults: update 2009.* Thorax, 2009. 64 Suppl 3: p. iii1-55.
39. Rosenvinge, F.S. *Antibiotikavejledning for Region Syddanmark.* 06.10.2021 [cited 2022 22 september]; Available from: https://ekstern.infonet.regionsyddanmark.dk/Files/Dokument547684.htm.
40. Plesner, L.L., et al., *The formation and design of the TRIAGE study-baseline data on 6005 consecutive patients admitted to hospital from the emergency department.* Scandinavian journal of trauma, resuscitation and emergency medicine, 2015. 23(1): p. 1-9.
41. *User Manuel Danish Emergency Process Triage*.
42. Nordberg, M., S. Lethvall, and M. Castrén, *The validity of the triage system ADAPT.* Scandinavian journal of trauma, resuscitation and emergency medicine, 2010. 18: p. 1-1.
43. Farrohknia, N., et al., *Emergency department triage scales and their components: a systematic review of the scientific evidence.* Scand J Trauma Resusc Emerg Med, 2011. 19: p. 42.
44. Jones, M., *NEWSDIG: The National Early Warning Score Development and Implementation Group.* Clin Med (Lond), 2012. 12(6): p. 501-3.
45. Htun, T.P., et al., *Clinical features for diagnosis of pneumonia among adults in primary care setting: A systematic and meta-review.* Sci Rep, 2019. 9(1): p. 7600.
46. Gong, L., et al., *Clinical profile analysis and nomogram for predicting in-hospital mortality among elderly severe community-acquired pneumonia patients with comorbid cardiovascular disease: a retrospective cohort study.* BMC Pulm Med, 2022. 22(1): p. 312.
47. Sakakibara, T., et al., *A prediction rule for severe adverse events in all inpatients with community-acquired pneumonia: a multicenter observational study.* BMC pulmonary medicine, 2022. 22(1): p. 34.
48. Mogensen, C.B., et al., *Ear measurement of temperature is only useful for screening for fever in an adult emergency department.* BMC Emerg Med, 2018. 18(1): p. 51.
49. Mackowiak, P.A., F.A. Chervenak, and A. Grünebaum, *Defining Fever.* Open Forum Infect Dis, 2021. 8(6): p. ofab161.
50. Waterer, G.W., L.A. Kessler, and R.G. Wunderink, *Medium-term survival after hospitalization with community-acquired pneumonia.* Am J Respir Crit Care Med, 2004. 169(8): p. 910-4.

51. Zhao, L.H., J. Chen, and R.X. Zhu, *The relationship between frailty and community-acquired pneumonia in older patients.* Aging Clin Exp Res, 2023. 35(2): p. 349-355.
52. Kitazawa, T., et al., *Characteristics of pneumonia with negative chest radiography in cases confirmed by computed tomography.* J Community Hosp Intern Med Perspect, 2020. 10(1): p. 19-24.
53. Huang, Y., et al., *Diagnostic value of blood parameters for community-acquired pneumonia.* Int Immunopharmacol, 2018. 64: p. 10-15.
54. Alzoubi, O. and A. Khanfar, *Association between neutrophil to lymphocyte ratio and mortality among community acquired pneumonia patients: a meta-analysis.* Monaldi Arch Chest Dis, 2021. 92(3).
55. Milas, G.P., V. Issaris, and V. Papavasileiou, *Blood urea nitrogen to albumin ratio as a predictive factor for pneumonia: A meta-analysis.* Respir Med Res, 2022. 81: p. 100886.
56. Kassaw, G., et al., *Outcomes and Predictors of Severe Community-acquired Pneumonia Among Adults Admitted to the University of Gondar Comprehensive Specialized Hospital: A Prospective Follow-up Study.* Infect Drug Resist, 2023. 16: p. 619-635.
57. Adnan, M., et al., *Prognostic value of five serum markers predicting in-hospital mortality among adults with community acquired pneumonia.* J Infect Dev Ctries, 2022. 16(1): p. 166-172.
58. Rendón-Ramirez, E.J., et al., *TGF-β Blood Levels Distinguish Between Influenza A (H1N1)pdm09 Virus Sepsis and Sepsis due to Other Forms of Community-Acquired Pneumonia.* Viral Immunol, 2015. 28(5): p. 248-54.
59. Watanabe, H., et al., *Clinical factors associated with negative urinary antigen tests implemented for the diagnosis of community-acquired pneumococcal pneumonia in adult patients.* Med Princ Pract, 2015. 24(2): p. 189-94.
60. Zeng, W., et al., *Association of admission blood glucose level and clinical outcomes in elderly community-acquired pneumonia patients with or without diabetes.* Clin Respir J, 2022. 16(8): p. 562-571.
61. Barmanray, R.D., et al., *In-hospital hyperglycemia but not diabetes mellitus alone is associated with increased in-hospital mortality in community-acquired pneumonia (CAP): a systematic review and meta-analysis of observational studies prior to COVID-19.* BMJ Open Diabetes Res Care, 2022. 10(4).

62. van der Meer, V., et al., *Diagnostic value of C reactive protein in infections of the lower respiratory tract: systematic review.* Bmj, 2005. 331(7507): p. 26.
63. van Vugt, S.F., et al., *Use of serum C reactive protein and procalcitonin concentrations in addition to symptoms and signs to predict pneumonia in patients presenting to primary care with acute cough: diagnostic study.* Bmj, 2013. 346: p. f2450.
64. Ebell, M.H., et al., *Accuracy of Biomarkers for the Diagnosis of Adult Community-acquired Pneumonia: A Meta-analysis.* Acad Emerg Med, 2020. 27(3): p. 195-206.
65. Ebell, M.H., et al., *Accuracy of Signs and Symptoms for the Diagnosis of Community-acquired Pneumonia: A Meta-analysis.* Acad Emerg Med, 2020. 27(7): p. 541-553.
66. Division of Nutrition, P.A., and Obesity, National Center for Chronic Disease Prevention and Health Promotion. 3 June 2022 [cited 2023 2 March]; Available from: https://www.cdc.gov/obesity/basics/adult-defining.html.

11.1.2 Paper I – TRIPOD Checklist



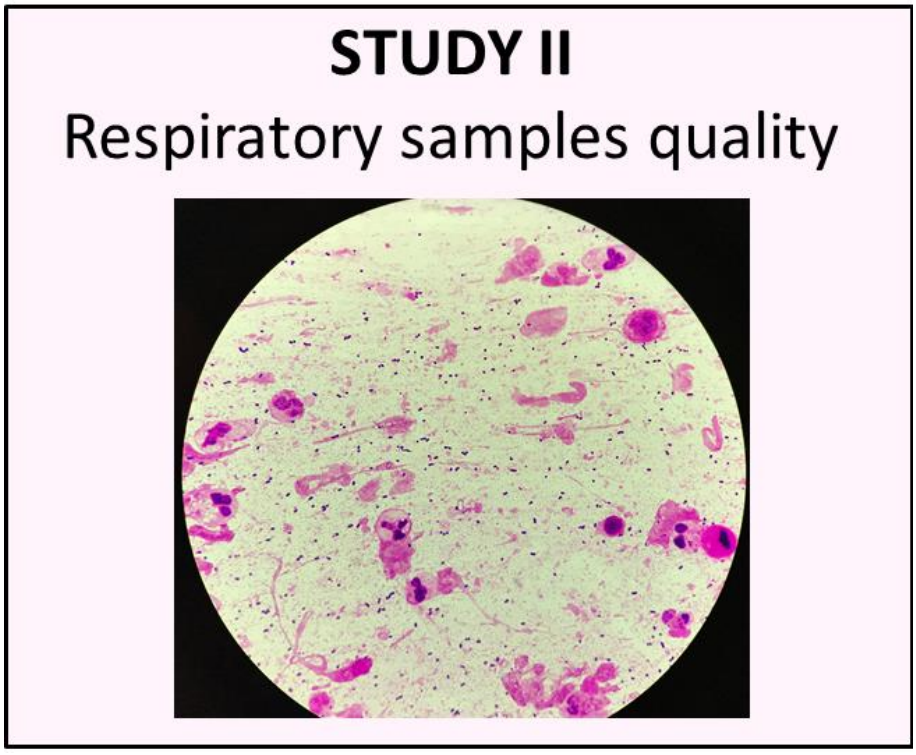
TRIPOD Checklist: Prediction Model Development and Validation

Section/Topic	Item		Checklist Item	Page
Title and abstract				
Title	1	D;V	Identify the study as developing and/or validating a multivariable prediction model, the target population, and the outcome to be predicted.	1
Abstract	2	D;V	Provide a summary of objectives, study design, setting, participants, sample size, predictors, outcome, statistical analysis, results, and conclusions.	2
Introduction				
Background and objectives	3a	D;V	Explain the medical context (including whether diagnostic or prognostic) and rationale for developing or validating the multivariable prediction model, including references to existing models.	3-4
	3b	D;V	Specify the objectives, including whether the study describes the development or validation of the model or both.	5
Methods				
Source of data	4a	D;V	Describe the study design or source of data (e.g., randomized trial, cohort, or registry data), separately for the development and validation data sets, if applicable.	5
	4b	D;V	Specify the key study dates, including start of accrual; end of accrual; and, if applicable, end of follow-up.	5
Participants	5a	D;V	Specify key elements of the study setting (e.g., primary care, secondary care, general population) including number and location of centres.	5
	5b	D;V	Describe eligibility criteria for participants.	6
	5c	D;V	Give details of treatments received, if relevant.	n/a
Outcome	6a	D;V	Clearly define the outcome that is predicted by the prediction model, including how and when assessed.	6-7
	6b	D;V	Report any actions to blind assessment of the outcome to be predicted.	7
Predictors	7a	D;V	Clearly define all predictors used in developing or validating the multivariable prediction model, including how and when they were measured.	7 + additional file (table S1 and S2)
	7b	D;V	Report any actions to blind assessment of predictors for the outcome and other predictors.	7
Sample size	8	D;V	Explain how the study size was arrived at.	8
Missing data	9	D;V	Describe how missing data were handled (e.g., complete-case analysis, single imputation, multiple imputation) with details of any imputation method.	9
Statistical analysis methods	10a	D	Describe how predictors were handled in the analyses.	9
	10b	D	Specify type of model, all model-building procedures (including any predictor selection), and method for internal validation.	9
	10c	V	For validation, describe how the predictions were calculated.	9

	10d	D;V	Specify all measures used to assess model performance and, if relevant, to compare multiple models.	9
	10e	V	Describe any model updating (e.g., recalibration) arising from the validation, if done.	9
Risk groups	11	D;V	Provide details on how risk groups were created, if done.	n/a
Development vs. validation	12	V	For validation, identify any differences from the development data in setting, eligibility criteria, outcome, and predictors.	
Results				
Participants	13a	D;V	Describe the flow of participants through the study, including the number of participants with and without the outcome and, if applicable, a summary of the follow-up time. A diagram may be helpful.	10
	13b	D;V	Describe the characteristics of the participants (basic demographics, clinical features, available predictors), including the number of participants with missing data for predictors and outcome.	10-12 (Table 1) + additional file (table S2)
	13c	V	For validation, show a comparison with the development data of the distribution of important variables (demographics, predictors and outcome).	additional file (table S3)
Model development	14a	D	Specify the number of participants and outcome events in each analysis.	10-14
	14b	D	If done, report the unadjusted association between each candidate predictor and outcome.	10-14(Table 1) + table 2
Model specification	15a	D	Present the full prediction model to allow predictions for individuals (i.e., all regression coefficients, and model intercept or baseline survival at a given time point).	14
	15b	D	Explain how to the use the prediction model.	14 + additional file (formula S6 +S7)
Model performance	16	D;V	Report performance measures (with CIs) for the prediction model.	14
Model-updating	17	V	If done, report the results from any model updating (i.e., model specification, model performance).	14
Discussion				
Limitations	18	D;V	Discuss any limitations of the study (such as nonrepresentative sample, few events per predictor, missing data).	17-18
Interpretation	19a	V	For validation, discuss the results with reference to performance in the development data, and any other validation data.	15-16
	19b	D;V	Give an overall interpretation of the results, considering objectives, limitations, results from similar studies, and other relevant evidence.	15+16+ 17
Implications	20	D;V	Discuss the potential clinical use of the model and implications for future research.	14+15+16+17
Other information				
Supplementary information	21	D;V	Provide information about the availability of supplementary resources, such as study protocol, Web calculator, and data sets.	4
Funding	22	D;V	Give the source of funding and the role of the funders for the present study.	19

*Items relevant only to the development of a prediction model are denoted by D, items relating solely to a validation of a prediction model are denoted by V, and items relating to both are denoted D;V. We recommend using the TRIPOD Checklist in conjunction with the TRIPOD Explanation and Elaboration document.

11.2 Paper II



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Article

Expiratory Technique versus Tracheal Suction to Obtain Good-Quality Sputum from Patients with Suspected Lower Respiratory Tract Infection: A Randomized Controlled Trial

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Abstract: Microbiological diagnostics of good-quality sputum samples are fundamental for infection control and targeted treatment of lower respiratory tract infections (LRTI). This study aims to compare the expiratory technique and tracheal suction on the quality of sputa from adults acutely hospitalized with suspected LRTI. We performed an open-label, randomized controlled trial. Patients were randomized to sputum sampling by tracheal suction (standard care) or the expiratory technique. The primary outcome was quality of sputum evaluated by microscopy and was analysed in the intention-to-treat population. The secondary outcomes were adverse events and patients experience. In total, 280 patients were assigned to tracheal suction (n = 141, 50.4%) or the expiratory technique (n = 139, 49.6%). Sputum samples were collected from 122 (86.5%) patients with tracheal suction and 67 (48.2%) patients with expiratory technique. Good-quality sputa were obtained more often with tracheal suction than with expiratory technique (odds ratio 1.83 [95% CI 1.05 to 3.19]; p = 0.035). There was no statistical difference in adverse events (IRR 1.21 [95% CI, 0.94 to 1.66]; p = 0.136), but patient experience was better in the expiratory technique group (p < 0.0001). In conclusion, tracheal suction should be considered a routine procedure in emergency departments for patients with suspected LRTI.

Keywords: lower respiratory tract infection; sputum; tracheal suction; forced expiratory technique; randomized controlled trial; emergency department

check for updates

Citation: Cartuliares, M.B.; Rosenvinge, F.S.; Mogensen, C.B.; Skovsted, T.A.; Andersen, S.L.; Pedersen, A.K.; Skjøt-Arkil, H. Expiratory Technique versus Tracheal Suction to Obtain Good-Quality Sputum from Patients with Suspected Lower Respiratory Tract Infection: A Randomized Controlled Trial. *Diagnostics* **2022**, *12*, 2504. https://doi.org/10.3390/diagnostics12102504

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1. Introduction

Lower respiratory tract infections (LRTI) are common infectious diseases, accounting for about three million global deaths every year [1]. Targeted antibiotic treatment based on precise diagnosis is essential to avoid antimicrobial overuse and the development of antibiotic resistance. In addition, a microbiological diagnosis can have important implications for patient management and infection control measures, highlighted by the current COVID-19 pandemic. Several clinical guidelines recommend collecting a sputum sample and adjusting treatment according to identified pathogens [2,3].

Even though sputum samples provide a guide for appropriate treatment [4–6], the usefulness of sputum samples has been questioned, primarily due to the difficulty in obtaining good-quality sputum samples [7,8].

Sputum samples can be collected by several methods [9–11]. However, these methods are poorly described, and most samples are collected by self-expectoration [5]. Tracheal suction (TS) is shown to reduce contamination from the microbiota in the upper airways and is more likely to detect infectious pathogens than expectorated sputa [12–14] as the microbiota from the upper airways may falsely indicate a pathogen from the LRT or may overgrow the actual pathogen decreasing the diagnostic yield in culture. However, low accuracy and misclassification have also been reported [15]. In addition, patients have described the TS as painful [16], and adverse events such as hypoxia, oxygen desaturation, and mucosal bleeding have been reported [17]. The forced expiratory technique (FET) is an instructed method to facilitate expectoration that can be combined with saline inhalation to induce sputum (FETIS) [9,18,19]. Induced sputum is shown to be useful in diagnosing pulmonary tuberculosis [20]. FETIS is reported to be safe and non-invasive, but hypertonic saline and prolonged inhalation have been associated with severe adverse effects [21,22]. Previous studies have compared the different techniques in specialized departments. Generally, TS is recommended for mechanically ventilated patients to reduce the risk of infections as they have mucus retention and difficulties to cough up secretions [17]; moreover, TS can contribute to unique information on etiological agents when obtained immediately after intubation in patients with severe community-onset pneumonia [23]. FETIS has been shown to result in better prognoses in patients with a wide range of chronic respiratory diseases including cystic fibrosis, bronchiectasis, and COPD [9,10]. In the acute setting, sputum samples have important diagnostic implications as both targeted antibiotic treatment and appropriate infection control measures rely on valid microbiological results [2,3]. Poor quality samples contaminated with oropharyngeal microbiota on the other hand may lead to misleading diagnostics and inappropriate use of antibiotics. Guidelines therefore recommended only accepting good-quality samples with a low concentration of squamous epithelium from the upper airways for microbiological diagnostics [24]. This clearly underlines the importance of using the most efficient sample method.

The effectiveness of TS compared with FETIS to obtain a good-quality sputum sample has not been investigated in an emergency department (ED) setting, where the majority of patients with LRTI are seen and where safe and fast procedures, not requiring advanced skills, are requested.

This randomized controlled trial aimed to test the hypothesis that FETIS was non-inferior to (not worse than) TS in collecting good-quality sputum samples from adult patients with suspected LRTI in an acute medical ward (primary outcome). As secondary outcomes, we compared adverse events and patient experiences.

2. Materials and Methods

2.1. Study Design and Setting

This study was designed as a single-centre, non-inferiority, open-label, randomized controlled trial. The trial was conducted at Hospital Sønderjylland, which comprises two emergency departments (Aabenraa and Sønderborg) with a hospital coverage of approximately 225.000 inhabitants. A Danish ED is equivalent to an acute medical ward. The study was reported in accordance with the Consolidation Standard of Reporting Trials (CONSORT) guidelines for parallel-group randomized trials [25]. The protocol was approved by the Regional Committee on Health Research Ethics for Southern Denmark (S-20200133), registered by the Danish Data Protection Agency (20/41767) and by ClinicalTrials.gov (NCT04595526) on 20 October 2020, and completed on 5 July 2021. The statistical analysis plan (SAP) and study protocol have been published, and this publication details further information about the trial methods [26].

2.2. Selection of Participants

Admitted patients with suspected LRTI were consecutively identified in the patient management system (CETREA 4.2.0.0.) at the ED by a project assistant. The attending physician confirmed eligibility, and the patient's verbal and written consent was obtained

by the project assistant. Adults (>18 years of age) admitted to the ED with suspected LRTI were enrolled in the study if the attending physician identified at least one of the following pulmonary symptoms: dyspnoea, cough, expectoration, chest pain, or fever. Patients were excluded if project enrolment and sputum collection would delay urgent, lifesaving treatment (e.g., in case of severe hypoxia or cardiac events) or transfer to an intensive care unit, or if the patient had severe immunodeficiency [26].

2.3. Randomization and Masking

Eligible patients were randomly assigned (1:1) to either TS procedure (usual care) or FETIS (intervention). Randomization was performed by project assistants using a computer-generated randomization tool (Research Electronic Data Capture) [27], prior to collection of the sputum sample. An independent data manager generated the sequence using random block sizes of six without stratification. The data manager had no further involvement in the study. In addition, the project assistants did not have access to the randomization code, sequence, or block sizes at any time during the trial.

The study was an open-label trial as masking the intervention from participants, project assistants, or outcome assessors was impossible in the clinical setting. The statistician was blinded until data analysis was completed.

2.4. Interventions

Six experienced project assistants from the ED identified eligible patients; collected informed consent and patient information; and received bedside and simulation training in FET, FETIS, and TS. Furthermore, a standardized protocol for performing FETIS was developed to support consistent data collection [26]. Sputum samples were collected from patients as soon as possible or within 24 h of admission. This criterion deviated from the study protocol that stated that samples would be collected within one hour. This deviation was due to the difficulty of collecting samples in this time frame in the clinical setting. TS was performed with catheter insertion into the nares during inhalation. The catheter was gently advanced about 40 cm into the trachea, where suctioning at 200–400 mmHg was performed before withdrawing the catheter [26]. FETIS was based on the patients' attempts to deliver a sputum sample and included FET alone and FET after sputum induction with isotonic inhalation. Efforts to minimize oropharyngeal contamination included rinsing the mouth and detailed, standard, verbal instructions in proper forced exhalation and coughing techniques [26]. Patients were instructed to deliver a sputum sample using FET. Regardless of the success of expectoration, sputum was induced using isotonic saline inhalation (0.9%) for 10 min [18], and the patient was once again instructed in FET (FETIS) [26]. Participants in the intervention group who could not deliver a sputum sample by FETIS underwent TS. These samples were not included in the intention-to-treat analysis.

2.5. Outcome Measures

The primary outcome was the quality of the sputum samples. The quality was defined as good or poor quality by Gram stain, and microscopy was described thoroughly in the study protocol [26]. Samples with <10 squamous epithelial cells per low power field of view (10× objective) were classified as good quality (illustrated in Figure 1), and samples with ≥10 squamous epithelial cells were classified as poor quality [28]. Sputum samples unable to be collected were considered missing as the quality could not be determined [26]. Gram stains were performed daily, and the results were generally available within 48 h.

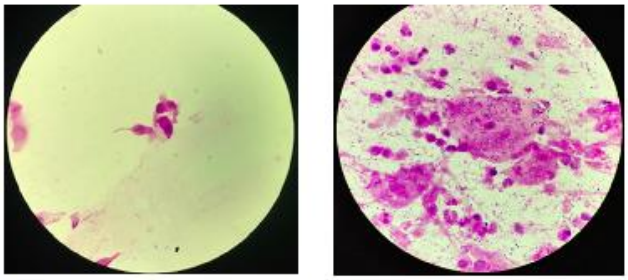

Figure 1. Example of Gram-stained good-quality specimens (×100 magnification). Cylindrical epithelial cell from tracheal suction (**left**) and expectorated sputum (**right**) (dense with polymorph nuclear leucocytes among Gram-positive diplococci and a single squamous epithelial cell with adhering microbiota from the upper respiratory tract). (Photo by Lomborg SA).

Secondary outcomes included adverse events and patients' experience of delivering the sputum sample. Pooled adverse events were reported and included seven variables measured before and within 10 min after sputum collection. The variables included (1) aggravation of oxygen saturation (SaO_2) ≤93% (Chronic Obstructive Pulmonary Disease SaO_2 ≤88%), (2) aggravation of respiratory rate (RR) ≤12/min or >20/min, (3) patient-reported aggravation of symptoms (cough, expectoration, dyspnoea, and chest pain), (4) aggravation of patient symptoms measured by Borg scale CR10, (5) occurrence of observed side effects, (6) mortality within a week, and (7) 30-day readmission. The published study protocol describes a more thorough explanation of these variables [26]. Immediately after sputum collection, the participants were requested to give a verbal score to the question: "What was your experience with this procedure?" using a five-point Likert scale ranging from "very bad", "bad", "neither bad nor good", "good", and "very good". A visual support tool describing this scoring system was available to assist patients. Finally, participants were asked to explain the reason behind their rating [26].

2.6. Statistical Analysis

Statistical methods and sample size calculations are described in the SAP and study protocol for the trial. The SAP was developed and submitted before completion of recruitment, database closure, and statistical analyses [26]. To estimate the sample size, we assumed a difference between the procedures of 15% (the pre-specified margin of primary outcome). In addition, we assumed 30% missed samples in the FETIS group and 10% in the TS group. With a two-sided *p*-value, an alpha level of 5%, and a power of 84%, we would need 260 patients equally distributed between the two groups. The primary analysis followed the intention-to-treat protocol and was repeated for sensitivity purposes as a complete case analysis. The primary outcome was analysed using logistic regression. An adjusted analysis was conducted to minimize the risk of Gail's bias [29]. Odds ratios (OR) and confidence intervals (CI) were reported. For the secondary outcomes, pooled adverse events and patient experience, we performed a Poisson regression and Wilcoxon test, respectively. Additionally, a sensitivity analysis for each type of adverse event was performed by either a chi-square test or Fischer's exact test. Agreement of the sputum quality between FET alone and FET after sputum induction with isotonic inhalation was assessed using κ-statistics. In addition, descriptive analyses were conducted on the numbers and quality of tracheal secretions for patients in the FETIS group that could not deliver an expectorated sputum. Analyses were performed using STATA 17.0 (TX, USA). During data collection, an external assessor supervised the performance of the project assistants, and an independent microbiology expert ensured the quality of the specimen data. The project

investigator monitored the daily inclusion of the patients, discussing as necessary progress with the study assistants and steering committee.

3. Results

In total, 534 patients were screened for eligibility between 10 November 2020 and 5 July 2021, of whom 280 (52.4%) underwent randomization. Patients were allocated to either the TS group (141 patients (50.4%)) or the FETIS group (139 patients (49.6%)) and comprised the intention-to-treat population. In the complete case analyses, 119 (85%) and 67 (48%) samples were included from the TS and FETIS groups, respectively (Figure 2).

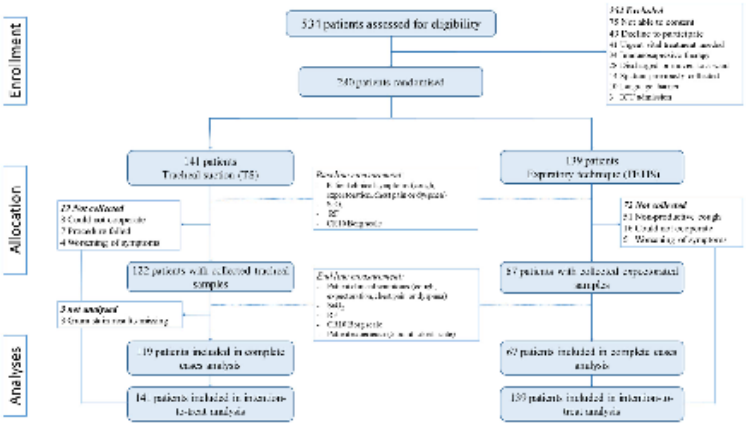

Figure 2. Trial profile. Randomization effectively created a balance between the two groups regarding demographic and clinical characteristics (Table 1).

Table 1. Baseline characteristics of the intention-to-treat population.

	TS (*n* = 141)	FETIS (*n* = 139)	Total (*n* = 280)
Hospital Sønderjylland			
ED in Aabenraa	116 (82%)	112 (81%)	228 (81%)
ED in Sønderborg	25 (18%)	27 (19%)	52 (18%)
Sex (male)	79 (56%)	82 (59%)	161 (58%)
Age, mean years	72.9 (12.3)	71.5 (12.7)	72.2 (12.5)
Nursing home resident	8 (6%)	4 (3%)	12 (4%)
Smoking status			
Non-smokers	38 (27%)	32 (23%)	70 (25%)
Ex-smokers	76 (54%)	83 (60%)	159 (57%)
Current smokers	26 (18%)	24 (17%)	50 (18%)
Length of hospital stay †, days	5.0 (2.1; 8.0)	4.0 (1.9; 6.9)	4.1 (2.0; 7.1)
SYMPTOMS			
Cough	86 (61%)	81 (58%)	167 (60%)
Expectoration	84 (60%)	77 (55%)	161 (58%)
Chest tightness	45 (32%)	49 (35%)	94 (34%)
Dyspnoea	96 (68%)	92 (66%)	188 (67%)

Table 1. *Cont.*

	TS (*n* = 141)	FETIS (*n* = 139)	Total (*n* = 280)
SEVERITY ASSESSMENT †			
CURB-65 *			
Mild 0–1	62 (50%)	67 (60%)	129 (53%)
Moderate 2	43 (34%)	42 (35%)	85 (35%)
Severe 3–5	20 (16%)	11 (9%)	31 (13%)
Triage **			
Triage level 1	8 (6%)	10 (7%)	18 (7%)
Triage level 2–3	99 (71%)	105 (76%)	204 (73%)
Triage level 4–5	33 (24%)	24 (17%)	57 (20%)
Suspicion of pneumonia	99 (70%)	100 (72%)	199 (71%)
SARS-CoV-2 positive	24 (17%)	16 (12%)	40 (14%)
COMORBIDITIES †			
Any comorbidity	128 (91%)	119 (86%)	247 (88%)
Respiratory diseases	66 (52%)	64 (55%)	130 (54%)
COPD ***	50 (36%)	53 (38%)	103 (37%)
Cardiovascular Diseases	85 (68%)	78 (68%)	163 (68%)
Neurological diseases	24 (19%)	25 (22%)	49 (20%)
DM ****	29 (21%)	32 (23%)	61 (22%)
Cancer	30 (21%)	23 (17%)	53 (19%)
Other diseases	60 (43%)	61 (44%)	121 (43%)
VITAL PARAMETERS			
Oxygen saturation, %	95.0 (93.0; 97.0)	96.0 (93.0; 98.0)	95.0 (93.0; 97.0)
Respiratory rate/min	21.0 (18.0; 24.0)	21.0 (18.0; 24.0)	21.0 (18.0; 24.0)
Heart rate/min	91.6 (21.6)	90.1 (17.3)	90.9 (19.6)
Systolic Blood pressure, mmHg	130.9 (20.8)	134.3 (22.6)	132.6 (21.8)
Diastolic blood pressure, mmHg	71.9 (14.5)	74.7 (16.4)	73.3 (15.5)
Fever > 38 °C	42 (30%)	45 (32%)	87 (31%)
Altered mental status	13 (10%)	9 (7%)	22 (8%)
BLOOD TESTS			
C-reactive protein, mg/L	74.0 (20.0; 168.0)	46.0 (16.0; 116.0)	54.0 (19.0; 149.0)
Leucocytes, 10^9/L	10.8 (8.0; 14.5)	10.4 (7.5; 14.1)	10.7 (7.9; 14.2)
Neutrophilocytes, 10^9/L	8.4 (5.9; 11.8)	7.9 (5.2; 11.0)	8.2 (5.5; 11.3)
ANTIBIOTIC TREATMENT			
Within one month	47 (33%)	48 (35%)	95 (34%)
Prior sputum collection	58 (41%)	56 (40%)	114 (41%)
Inhaled medications	33 (23%)	38 (27%)	71 (25%)

Data are *n* (%), median (IQR), or mean (SD). * CURB-65: Confusion, Urea, Respiratory rate, Blood pressure and age > 65 [26]. ** Triage: Danish Emergency Process Triage (DEPT) [26]. *** COPD: Chronic obstructive pulmonary disease. **** DM: Diabetes Mellitus I or II. † Data not available for all randomized patients.

The intention-to-treat analysis showed that the chance of obtaining a good-quality sputum sample was significantly higher using TS rather than FETIS (OR 1.83 [95% CI, 1.05 to 3.19]; $p = 0.035$) (Table 2). For the complete case analysis, the OR was 2.42 [95% CI, 1.31 to 4.47]; $p = 0.005$. The sensitivity and sub-analyses are described in the Supplementary Materials, Tables S1 and S2. The difference between groups was 15.06% points (58.82% for TS and 43.76% for the intervention group). There was no statistical difference when comparing the number of pooled adverse events between groups (IRR 1.21 [95% CI, 0.94 to 1.66]; $p = 0.136$) (Table 2). The sensitivity analysis showed no difference between any particular adverse event in the two groups except for bleeding ($p = 0.0002$) and dyspnoea (0.034) (Supplementary Materials, Table S3). Adverse events were reported as mild, short-lived, and without need for blood transfusions or physician consultation, except for one patient where the bleeding was reported as moderate and required physician consultation (Supplementary Materials, Text S1). There was a statistically significant difference in how patients experienced sputum collection. Patients from the FETIS group generally reported

a better experience than those randomised to TS ($p < 0.0001$) (Table 2 and Supplementary Materials, Figure S1 and Table S4). Missing data for the secondary outcome were minimal, so no imputation was necessary. The TS group had 2% and 5% missing variables for mortality and readmission, respectively, and the FETIS group had 4% missing for readmission, and both groups had 1% missing for the reported Likert scale (Supplementary Materials, Table S3 and Figure S1). Of the 67 (48%) patients who delivered an expectorated sputum in the FETIS group, 41 (61%) produced two samples, 9 (13%) delivered a specimen only by FET, and 17 (25%) only produced a specimen by FET after sputum induction. Kappa statistics demonstrated agreement in the quality of sputum samples between FET alone (12 patients (24%)) and FET after sputum induction with isotonic inhalation (20 patients (34%)) (Kappa 0.99). A descriptive analysis of the 57 patients who could not deliver a sputum sample in the FETIS group can be seen in the Supplementary Materials (Figure S2 and Table S5). None of the sensitivity analyses questioned the robustness of the primary results, and the variance componence yielded negligible heterogeneity.

Table 2. Results from the quality of specimens collected by FETIS and TS procedures (intention-to-treat analysis) and from adverse effects and patient experience (complete case) with FETIS as a reference group for all analyses.

Primary Outcome	Unadjusted OR (95% CI)	*p*-Value
Quality of sputum samples	1.83 (1.05; 3.19)	0.035
Secondary Outcome	**Unadjusted IRR (95% CI)**	***p*-Value**
Adverse effects	1.21 (0.94; 1.66)	0.136
Patient experience of sputum collection procedure	N/A	<0.0001

4. Discussion

This study is the first randomised controlled trial comparing the quality of sputum samples collected by TS and FETIS. The result did not support our hypothesis and we showed that FETIS was inferior to TS, and TS had almost double the likelihood of ensuring a good-quality specimen. There were no differences in pooled adverse events, but FETIS was generally a more positive experience for patients than TS. The major challenge in sputum sample collection is the number of patients unable to deliver a good-quality sample [4,5,7]. This was also observed in our study, where only half of our patients delivered a sample using FETIS and less than half of these samples were of good quality despite efforts to improve expectoration.

Many studies focus on the usefulness of sputum in determining causative pathogens of LRTI but often fail to describe the sputum collection procedure adequately [5,7,30]. Different expiratory techniques positively affect secretion clearance, particularly for chronic conditions [9,10]. However, in our acute setting, FETIS did not facilitate easier secretion clearance or better-quality sputum samples. A systematic review reported that patients under instructed supervision during sputum collection delivered samples with better diagnostic value assessed by microscopy than uninstructed and unsupervised patients [31]. Therefore, our study prioritized supervision during FETIS with standardized protocols, experienced staff, and bedside training. Despite these efforts, the expiratory technique still produced sputum samples of inferior quality compared to TS.

The effectiveness of a saline solution in inducing sputum may vary with the concentration and duration of inhalation. The procedure is considered safe, with adverse events rarely reported [18]. A retrospective study focusing on patients with community-acquired pneumonia reported that a 3% saline inhalation for 30 min assisted the delivery of a quality specimen [19]. In contrast, an RCT including patients with a productive cough reported that inhalation with 3% saline for 10–15 min gave no improvement in the quality of the specimen compared to spontaneous cough [32]. The inhalation of hypertonic saline for 30–40 min was associated with dyspnoea, nausea, vomiting, and bronchoconstriction, and patients described the procedure as unpleasant, indicating a preference for bronchoscopy

rather than sputum induction [33]. In our study, the low concentration of the saline solution and the duration of inhalation (≤10 min) may have contributed to the few adverse events and the low number of patients describing the procedure as unpleasant. We chose a low concentration of saline inhalation for safety and tolerance reasons [18,21], but on the other hand, a higher concentration of saline inhalation has been used in other studies resulting in more collected samples [20]; however, these studies do not compare TS and FETIS sputum sample quality. The acute setting may have played a role in the challenges of collecting sputum by FETIS such as the inclusion of patients with severe co-morbidity or unproductive coughs or, alternatively, by the short duration of inhalation or low concentration of the saline solution.

It has previously been reported that there is less contamination from the upper respiratory microbiota when specimens are collected with TS [12–14]. However, these studies only focus on the quality of the sputum sample and do not randomize patients or describe the collection procedure in detail. Therefore, it is difficult to determine if the quality of expectorated sputa is inferior due to the collection procedure, population, or other factors. TS is a more invasive procedure, and adverse events such as hypoxia, oxygen desaturation, arrhythmia, and mucosal bleeding have been reported for mechanically ventilated patients [17]. In addition, ICU patients report discomfort and mild pain during suctioning [16]. In our study, patients randomized to the TS group had TS-related bleedings that were assessed as minor and short-lived, confirming that pain and discomfort related to TS are the most common reasons patients report negative experiences with TS. In our study, most patients had a neutral response on the Likert scale to either FETIS or TS, and the mean difference between groups was less than one (possible type 1 error). This result may reflect that patients may be willing to undergo tracheal suction in a clinical setting despite the risk of adverse events. This study represents patients classified with mild to moderate infection according to PSI, CURB-65, and Triage. International guidelines recommend sputum collection from patients with severe LRTI [2,3], a population excluded to some degree from our study. If we extrapolate the results from this study, a routine TS procedure for frail acute patients may reduce the number of sputum sample failures.

This study aimed to investigate the efficacy of expiratory techniques regardless of the patient's ability to expectorate. Therefore, some patients with a productive cough were allocated to the TS group, while others, unable to expectorate, were randomly assigned to the FETIS group. This random allocation is an important factor in the number of missing samples from the FETIS group. An alternative could be a multiple sample design whereby patients randomly attempt to deliver sputum samples by all three methods (FET, FETIS, and TS). However, this presents ethical challenges and may limit the generalizability of the study in an ED setting.

In contrast to other inferiority studies, we included two-sided *p*-values and CI, providing the true difference between the methods and minimizing sampling bias. There was no difference in pooled adverse events when comparing TS and FETIS, but FETIS was associated with a better patient experience but was clearly less effective in providing good-quality sputum samples supported by both the intention-to-treat and complete case analysis. Therefore, in a clinical setting, experts should not exchange TS with FETIS regardless of the benefits offered by the procedure.

The major strength of this study was the randomized controlled design, which enables us to compare the two sputum collection methods, minimizing confounding as much as possible. The variance between the staff collecting the samples was minimal with negligible heterogeneity. In addition, the standardized protocols, instructions, external supervision of the personnel, and quality monitoring of sputum samples ensured uniform data collection and increased the trial's internal validity. These factors increase the possibility that methods and procedures are applicable to other ED contexts.

A major limitation of this study was the open-label design of the trial. However, it was not possible to blind patients, project assistants, or technicians from the two procedures. Another potential limitation of our study was the high number of patients treated with an-

tibiotics before sampling. The diagnostic yield of sputum analysis decreases if patients have been treated with antibiotics [13,32], which has led to a debate questioning the usefulness of sputum collection. However, antibiotic treatment was evenly distributed between the TS and FETIS groups and the assessment of sputum quality based on identifying respiratory epithelial cells is not likely affected by antibiotic treatment. The decreased sensitivity of culture analysis in patients treated with antibiotics may be less of a problem when using polymerase chain reaction including multiplex, syndromic tests to diagnose LRTI, and antibiotic treatment is not likely to affect the detection of viral pathogens. However, like culture and microscopy, PCR is sensitive to contamination with upper respiratory microbiota, highlighting the importance of suiTable Samples. We did not measure other outcomes such as the amount of sputum or use forced expiratory volume (FEV_1) to monitor adverse events, as recommended and assessed in other studies [21,22]. However, our study focuses on the quality of the sputum as a prerequisite to culture and further diagnostics and not airway clearance and therapy, as these studies suggest. In our setting, we did not measure FEV_1 routinely when treating patients with acute LRTI, and the goal was a study design that reflected clinical practice.

5. Conclusions

Systematic reviews state that sputum samples of good quality are essential to identifying the aetiology of LRTI. In addition, clinical guidelines recommend good-quality sputum samples to support accurate LRTI diagnostics [2–5]. This study was is the first randomized controlled trial comparing the effectiveness of forced expiratory technique and tracheal suction on the quality of collected sputa in an emergency department setting. It gives useful insights into the optimal procedure to ensure the collection of good-quality sputum samples. We concluded that the forced expiratory technique is less likely to result in good-quality specimens and, therefore, is inferior to tracheal suction. In clinical practice, the implementation of TS in EDs might improve the likelihood of a correct diagnosis and the accurate treatment of LRTI. Further studies should consider multicentre locations and comparisons with other expiratory techniques and should investigate the microbiological yield from the two methods. TS should be considered a routine procedure in an ED context due to the limited value of FETIS in providing good-quality sputa, which is necessary for diagnosing LRTI.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/diagnostics12102504/s1, Table S1: Intention to treat analysis. Table S2: Complete cases. Table S3: Sensitivity analysis of adverse events. Text S1: Harms. Figure S1 and Table S4: Sensitivity analysis of the patient experience of the sputum collection procedure. Figure S2 and Table S5: Descriptive analysis from tracheal suctions obtained from patients unable to expectorate.

Author Contributions: M.B.C., F.S.R., C.B.M., T.A.S., S.L.A., A.K.P. and H.S.-A. were involved in the design of the study. M.B.C. performed the literature search and drafted the original work in collaboration with H.S.-A. M.B.C. was the study investigator and contributed to coordination responsibility, participant recruitment, and data collection. A.K.P. performed the statistical analyses. H.S.-A. was the research chief and responsible for supervision of the study. S.L.A. contributed substantially to the data quality and monitoring of the sputum samples. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Regional Committee on Health Research Ethics for Southern Denmark (S-20200133) approved 22 September 2020).

Informed Consent Statement: Informed consent was obtained both in writing and orally from all participants included in the study.

Data Availability Statement: Due to Danish laws on personal data, data cannot be shared publicly. To request these data, please contact the corresponding author for more information. The person responsible for the research was the principal investigator and corresponding author (M.B.C.), who together with the Department of Health Research and the University Hospital of Southern Denmark, owns the data and has access to the final data set.

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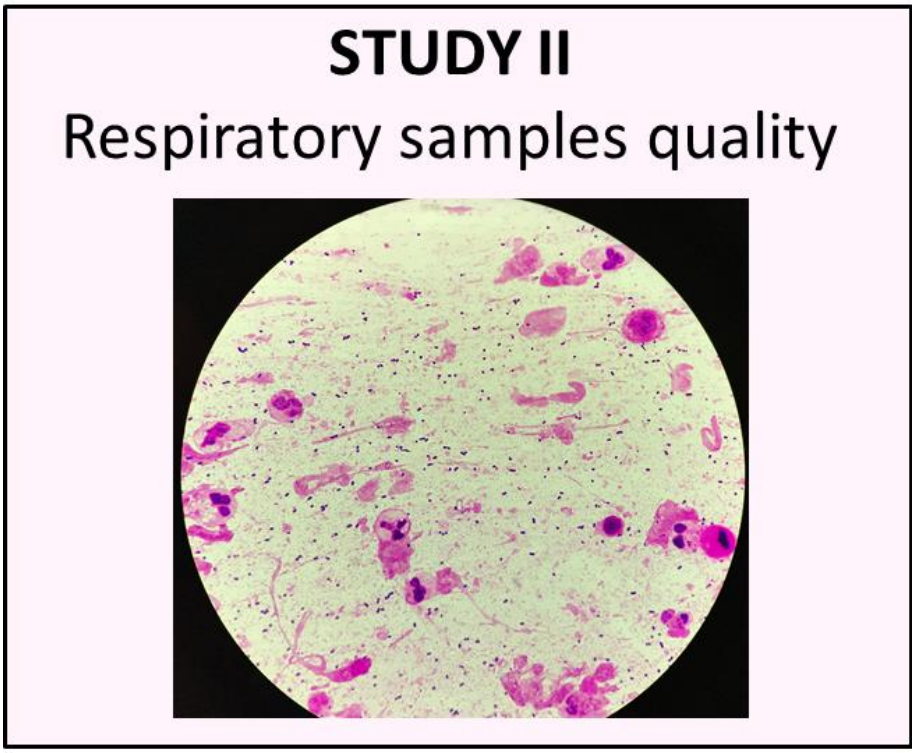
References

1. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990- 2016: A systematic analysis for the Global Burden of Disease Study 2016. *Lancet Infect. Dis.* **2018**, *18*, 1191–1210. [CrossRef]
2. Metlay, J.P.; Waterer, G.W.; Long, A.C.; Anzueto, A.; Brozek, J.; Crothers, K.; Cooley, L.A.; Dean, N.C.; Fine, M.J.; Flanders, S.A. Diagnosis and treatment of adults with community-acquired pneumonia. An official clinical practice guideline of the American Thoracic Society and Infectious Diseases Society of America. *Am. J. Respir. Crit. Care Med.* **2019**, *200*, e45–e67. [CrossRef] [PubMed]
3. Eccles, S.; Pincus, C.; Higgins, B.; Woodhead, M. Diagnosis and management of community and hospital acquired pneumonia in adults: Summary of NICE guidance. *BMJ* **2014**, *349*, g6722. [CrossRef] [PubMed]
4. Del Rio-Pertuz, G.; Gutiérrez, J.F.; Triana, A.J.; Molinares, J.L.; Robledo-Solano, A.B.; Meza, J.L.; Ariza-Bolivar, O.M.; Acosta-Reyes, J.; Garavito, A.; Viasus, D.; et al. Usefulness of sputum gram stain for etiologic diagnosis in community-acquired pneumonia: A systematic review and meta-analysis. *BMC Infect. Dis.* **2019**, *19*, 403. [CrossRef]
5. Ogawa, H.; Kitsios, G.D.; Iwata, M.; Terasawa, T. Sputum Gram Stain for Bacterial Pathogen Diagnosis in Community-acquired Pneumonia: A Systematic Review and Bayesian Meta-analysis of Diagnostic Accuracy and Yield. *Clin. Infect. Dis.* **2020**, *71*, 499–513. [CrossRef]
6. Roson, B.; Carratala, J.; Verdaguer, R.; Dorca, J.; Manresa, F.; Gudiol, F. Prospective study of the usefulness of sputum Gram stain in the initial approach to community-acquired pneumonia requiring hospitalization. *Clin. Infect. Dis.* **2000**, *31*, 869–874. [CrossRef]
7. Garcia-Vazquez, E.; Marcos, M.A.; Mensa, J.; de Roux, A.; Puig, J.; Font, C.; Francisco, G.; Torres, A. Assessment of the usefulness of sputum culture for diagnosis of community-acquired pneumonia using the PORT predictive scoring system. *Arch. Intern. Med.* **2004**, *164*, 1807–1811. [CrossRef]
8. Ewig, S.; Schlochtermeier, M.; Goke, N.; Niederman, M.S. Applying sputum as a diagnostic tool in pneumonia: Limited yield, minimal impact on treatment decisions. *Chest* **2002**, *121*, 1486–1492. [CrossRef]
9. Lewis, L.K.; Williams, M.T.; Olds, T.S. The active cycle of breathing technique: A systematic review and meta-analysis. *Respir. Med.* **2012**, *106*, 155–172. [CrossRef]
10. Andrews, J.; Sathe, N.A.; Krishnaswami, S.; McPheeters, M.L. Nonpharmacologic airway clearance techniques in hospitalized patients: A systematic review. *Respir. Care* **2013**, *58*, 2160–2186. [CrossRef]
11. Overend, T.J.; Anderson, C.M.; Brooks, D.; Cicutto, L.; Keim, M.; McAuslan, D.; Nonoyama, M. Updating the evidence base for suctioning adult patients: A systematic review. *Can. Respir. J.* **2009**, *16*, e6–e17. [CrossRef] [PubMed]
12. Geckler, R.W.; Gremillion, D.H.; McAllister, C.K.; Ellenbogen, C. Microscopic and bacteriological comparison of paired sputa and transtracheal aspirates. *J. Clin. Microbiol.* **1977**, *6*, 396–399. [CrossRef] [PubMed]
13. Gunasekaran, J.; Saksena, R.; Jain, M.; Gaind, R. Can sputum gram stain be used to predict lower respiratory tract infection and guide empiric antimicrobial treatment: Experience from a tertiary care hospital. *J. Microbiol. Methods* **2019**, *166*, 105731. [CrossRef] [PubMed]
14. Nagendra, S.; Bourbeau, P.; Brecher, S.; Dunne, M.; LaRocco, M.; Doern, G. Sampling variability in the microbiological evaluation of expectorated sputa and endotracheal aspirates. *J. Clin. Microbiol.* **2001**, *39*, 2344–2347. [CrossRef]
15. Tetenta, S.; Metersky, M.L. Tracheal aspirate Gram stain has limited sensitivity and specificity for detecting Staphylococcus aureus. *Respirology* **2011**, *16*, 86–89. [CrossRef]
16. Arroyo-Novoa, C.M.; Figueroa-Ramos, M.I.; Puntillo, K.A.; Stanik-Hutt, J.; Thompson, C.L.; White, C.; Wild, L.R. Pain related to tracheal suctioning in awake acutely and critically ill adults: A descriptive study. *Intensive Crit. Care Nurs.* **2008**, *24*, 20–27. [CrossRef]
17. Van de Leur, J.P.; Zwaveling, J.H.; Loef, B.G.; Van der Schans, C.P. Endotracheal suctioning versus minimally invasive airway suctioning in intubated patients: A prospective randomised controlled trial. *Intensive Care Med.* **2003**, *29*, 426–432. [CrossRef]
18. Chanez, P.; Holz, O.; Ind, P.; Djukanović, R.; Maestrelli, P.; Sterk, P. Sputum induction. *Eur. Respir. J.* **2002**, *20*, 3s–8s.

19. Bandyopadhyay, T.; Gerardi, D.A.; Metersky, M.L. A comparison of induced and expectorated sputum for the microbiological diagnosis of community acquired pneumonia. *Respiration* **2000**, *67*, 173–176. [CrossRef]
20. Butov, D.; Feshchenko, Y.; Myasoedov, V.; Kuzhko, M.; Gumeniuk, M.; Gumeniuk, G.; Tkachenko, A.; Nataliya, N.; Borysova, O.; Butova, T. Effectiveness of inhaled hypertonic saline application for sputum induction to improve Mycobacterium tuberculosis identification in patients with pulmonary tuberculosis. *Wien. Med. Wochenschr.* **2022**, *172*, 261–267. [CrossRef]
21. Pizzichini, M.; Leigh, R.; Djukanović, R.; Sterk, P. Safety of sputum induction. *Eur. Respir. J.* **2002**, *20*, 9s–18s.
22. Herrero-Cortina, B.; Alcaraz, V.; Vilaró, J.; Torres, A.; Polverino, E. Impact of hypertonic saline solutions on sputum expectoration and their safety profile in patients with bronchiectasis: A randomized crossover trial. *J. Aerosol Med. Pulm. Drug Deliv.* **2018**, *31*, 281–289. [CrossRef] [PubMed]
23. McCauley, L.M.; Webb, B.J.; Sorensen, J.; Dean, N.C. Use of Tracheal Aspirate Culture in Newly Intubated Patients with Community-Onset Pneumonia. *Ann. Am. Thorac. Soc.* **2016**, *13*, 376–381. [CrossRef] [PubMed]
24. Miller, J.M.; Binnicker, M.J.; Campbell, S.; Carroll, K.C.; Chapin, K.C.; Gilligan, P.H.; Gonzalez, M.D.; Jerris, R.C.; Kehl, S.C.; Patel, R. A guide to utilization of the microbiology laboratory for diagnosis of infectious diseases: 2018 update by the Infectious Diseases Society of America and the American Society for Microbiology. *Clin. Infect. Dis.* **2018**, *67*, e1–e94. [CrossRef]
25. Moher, D.; Hopewell, S.; Schulz, K.F.; Montori, V.; Gøtzsche, P.C.; Devereaux, P.J.; Elbourne, D.; Egger, M.; Altman, D.G. CONSORT 2010 explanation and elaboration: Updated guidelines for reporting parallel group randomised trials. *BMJ* **2010**, *340*, c869. [CrossRef] [PubMed]
26. Cartuliares, M.B.; Skjøt-Arkil, H.; Rosenvinge, F.S.; Mogensen, C.B.; Skovsted, T.A.; Pedersen, A.K. Effectiveness of expiratory technique and induced sputum in obtaining good quality sputum from patients acutely hospitalized with suspected lower respiratory tract infection: A statistical analysis plan for a randomized controlled trial. *Trials* **2021**, *22*, 675. [CrossRef]
27. Harris, P.A.; Taylor, R.; Minor, B.L.; Elliott, V.; Fernandez, M.; O'Neal, L.; McLeod, L.; Delacqua, G.; Delacqua, F.; Kirby, J.; et al. The REDCap consortium: Building an international community of software platform partners. *J. Biomed. Inform.* **2019**, *95*, 103208. [CrossRef]
28. Murray, P.R.; Washington, J.A. Microscopic and bacteriologic analysis of expectorated sputum. *Mayo Clin. Proc.* **1975**, *50*, 339–344.
29. Möller, S.; Bliddal, M.; Rubin, K.H. Methodical considerations on adjusting for Charlson Comorbidity Index in epidemiological studies. *Eur. J. Epidemiol.* **2021**, *36*, 1123–1128. [CrossRef]
30. Anevlavis, S.; Petroglou, N.; Tzavaras, A.; Maltezos, E.; Pneumatikos, I.; Froudarakis, M.; Anevlavis, E.; Bouros, D. A prospective study of the diagnostic utility of sputum Gram stain in pneumonia. *J. Infect.* **2009**, *59*, 83–89. [CrossRef]
31. Datta, S.; Shah, L.; Gilman, R.H.; Evans, C.A. Comparison of sputum collection methods for tuberculosis diagnosis: A systematic review and pairwise and network meta-analysis. *Lancet Glob. Health* **2017**, *5*, e760–e771. [CrossRef]
32. Chuard, C.; Fracheboud, D.; Regamey, C. Effect of sputum induction by hypertonic saline on specimen quality. *Diagn. Microbiol. Infect. Dis.* **2001**, *39*, 211–214. [CrossRef]
33. Miller, R.F.; Kocjan, G.; Buckland, J.; Holton, J.; Malin, A.; Semple, S.J. Sputum induction for the diagnosis of pulmonary disease in HIV positive patients. *J. Infect.* **1991**, *23*, 5–15. [CrossRef]

11.2.1 Paper II – Supplementary material



Supplementary Material - Sensitivity and sub-analyses

Expiratory technique versus tracheal suction to obtain good quality sputum from patients with suspected lower respiratory tract infection: a randomized controlled trial

Cartuliares MB, Rosenvinge FS, Mogensen CB, Skovsted TA, Andersen SL, Pedersen AK, Skjøt-Arkil H.

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I) Intention to treat analysis

Outcome	Unadjusted	p-value	Adjusted	p-value
Sputum quality	OR 1.83 (95%CI 1.05 to 3.19)	0 .035	OR 1.45 (95% CI 0.78 to 2·70)	0.233

Table S1: *Intention to treat analysis unadjusted and adjusted for antibiotics, pneumonia, smoking, SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2), CURB-65 (Confusion, Urea, Respiratory rate, Blood pressure and age >65), and age.*

II) Complete case analysis

Outcome	Unadjusted	p-value	Adjusted	p-value
Sputum quality	OR 2.42 (95%CI 1.31 to 4.47)	0.005	OR 1.96 (95%CI 0.98 to 3.90)	0.055
Adverse effects	IRR 1.02 (95%CI 0.87 to 1.19)	0.796	IRR 0.99 (95%CI 0.79 to 1.25)	0.985
Patient experience	N/A	< 0·001	N/A	< 0.001

Table S2: *Complete case analysis unadjusted and adjusted for antibiotics, pneumonia, smoking, SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2), CURB-65 (Confusion, Urea, Respiratory rate, Blood pressure and age >65), and age.*

III) Sensitivity analysis of adverse events

Adverse Events	TS		FETIS		p-value
	Total, n	Event, n (%)	Total, n	Event, n (%)	
Vital parameters					
Oxygen saturation*	141	21 (15%)	139	26 (19%)	0.426
Respiratory rate**	141	5 (4%)	139	3 (2%)	0.722
Side effects					
Procedure related bleeding	141	16 (11%)	139	1 (0·7%)	0 .0002†
Bronchospasm	141	3 (2%)	139	2 (1%)	1.000
Others***	141	5 (4%)	139	2 (1%)	0 .447
Patients symptoms					
Cough	141	6 (4%)	139	7 (5%)	0.784
Dyspnea	141	18 (13%)	139	7 (5%)	0 .034†
Chest tightness	141	3 (2%)	139	6 (4%)	0.333
Sputum	141	6 (4%)	139	9 (7%)	0 .439
CR 10 Borg scale					
CR10 report	141	22 (16%)	139	16 (12%)	0 .383
Mortality	138	2 (1%)	139	4 (3%)	0 .684
Readmission	134	40 (28%)	134	34 (25%)	0 .494

Table S3*: Sensitivity analysis of each adverse event included in pooled adverse event analysis. Number=n and percentage (%).*

Fishers Exact and χ^2 test was used to compare the adverse event variables.

Adverse events were reported during and at the latest 10 min after the procedure.

Aggravation of vital parameters: *Oxygen saturation decreased to ≤ 93% (Chronic Obstructive Pulmonary Disease patients ≤ 88%), **Respiratory rate decreased to ≤ 12 or increased to higher than 20 times per minute. Patient reported aggravation of symptoms measured by each symptom (cough, dyspnea, chest tightness and sputum) and measured by Borg scale CR10. Mortality was measured within 7 days from admission and readmission within one month from discharge.

***Others adverse events reported were nausea and vomiting.

† p-value significant < .05

IV) Harms (text S4)

After the analyses, the principal investigator reviewed all medical records for patients that experienced procedure-related bleedings, dyspnea, bronchospasms, decrease in oxygen saturation, and worsening of respiratory rate. This review ensured agreement between the project data and descriptions in the patient's medical record. An infectious disease and emergency medicine expert was consulted if there were discrepancies. Even though there was a difference between groups regarding bleedings and dyspnea, these adverse events were reported as mild, short-lived without the need for physician consultation except for one case described in detail below. In addition, worsening related oxygen saturation and respiratory rate were short-lived in patients treated with oxygen treatment and delivered a sample without oxygen.

Aggravation of dyspnea, decrease in oxygen saturation and bleeding:

One critically ill patient who was allocated the tracheal suction group experienced several symptoms at once (bleeding, dyspnea, oxygen desaturation) and consultation with a senior physician was required. The specialist in infectious disease and emergency medicine expert reviewed this medical record and assessed the patient's condition as critical from arrival. The worsening of the patient's vital parameters was not deemed a consequence of the tracheal suction procedure.

Bleeding:

One patient allocated to the intervention group and was unable to produce expectorate. This patient underwent tracheal suction and experienced bleeding from the nose and mouth in moderate severity and needed supervision until the condition was stabilized.

Bronchospasm:

Two patients experienced mild bronchospasms possibly due to treatment with b2-agonist prior to saline inhalation. One patient had no comorbidity and the other was a patient with chronic respiratory disease and a history of asthma. One patient in the intervention group required additional treatment 10 minutes after sputum induction due to a more severe bronchospasm.

V) Sensitivity analysis of the patient experience of the sputum collection procedure

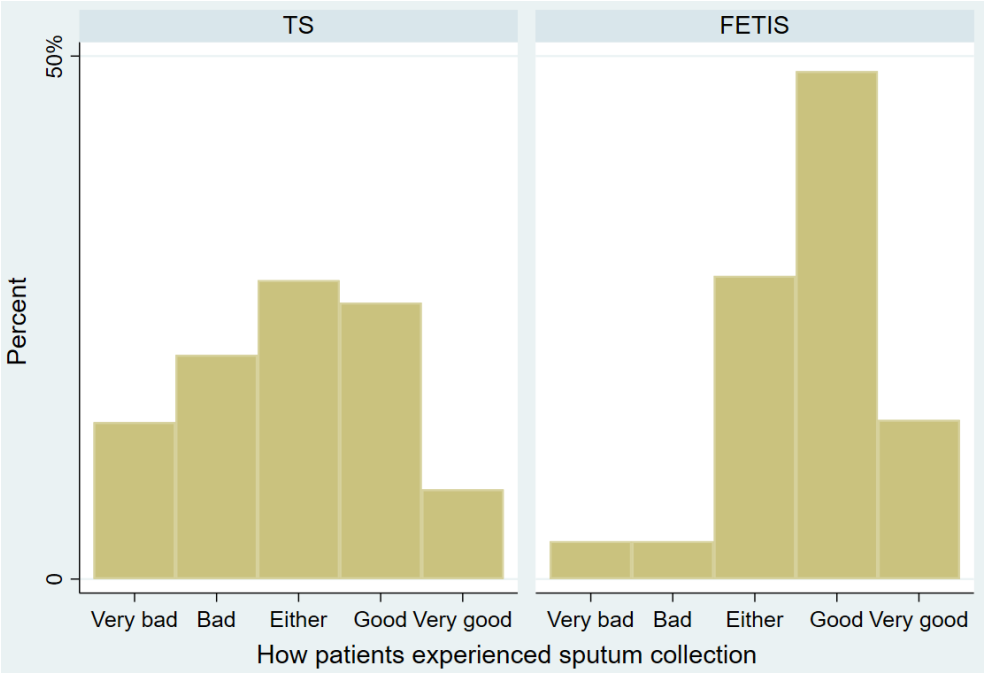


Figure S5: Distribution from Likert scale of patients experience. Comparison between the two groups of how patients experienced sputum collection p< 0.001 whereby 140 (99%) patients in the TS group and 138 (99%) from the intervention group answered the question.

Even though there were significant differences between groups p<0.0001, there was only a slight difference between groups of the neutral answer where the mean difference between groups was less than one 2.9 for TS and 3.6 for FETIS group.

Collecting method	TS (102)	FETIS (82)
Likert scale		
Very bad	21 (81%)	5(19%)
Bad	30 (86%)	5 (14%)
Either bad or good	40 (50%)	40 (50%)
Good	37 (36%)	67 (64%)
Very good	12 (36%)	21 (64%)
Explanations*	91 (55%)	74 (45%)
Painful and unpleasant	20 (22%)	4 (5%)
Breathless	6 (7%)	0 (0%)
Ineffective	2 (2%)	13 (18%)
Quickly	25 (27%)	2 (3%)
Acceptable	17 (19%)	21 (28%)
Easier breathing	5 (5%)	10 (13%)
Facilitate expectoration	0 (0%)	7 (9%)
Good information and a professional approach	10 (11%)	7 (9%)
Important for further treatment	6 (7%)	1 (2%)
Enjoyable	0 (0%)	9 (12%)

Table S6: *Patient's explanation of their choice from Likert scale*

*Patients can contribute with more than one explanation based on Likert scale

VI) Descriptive analysis from tracheal suctions obtained from patients unable to expectorate

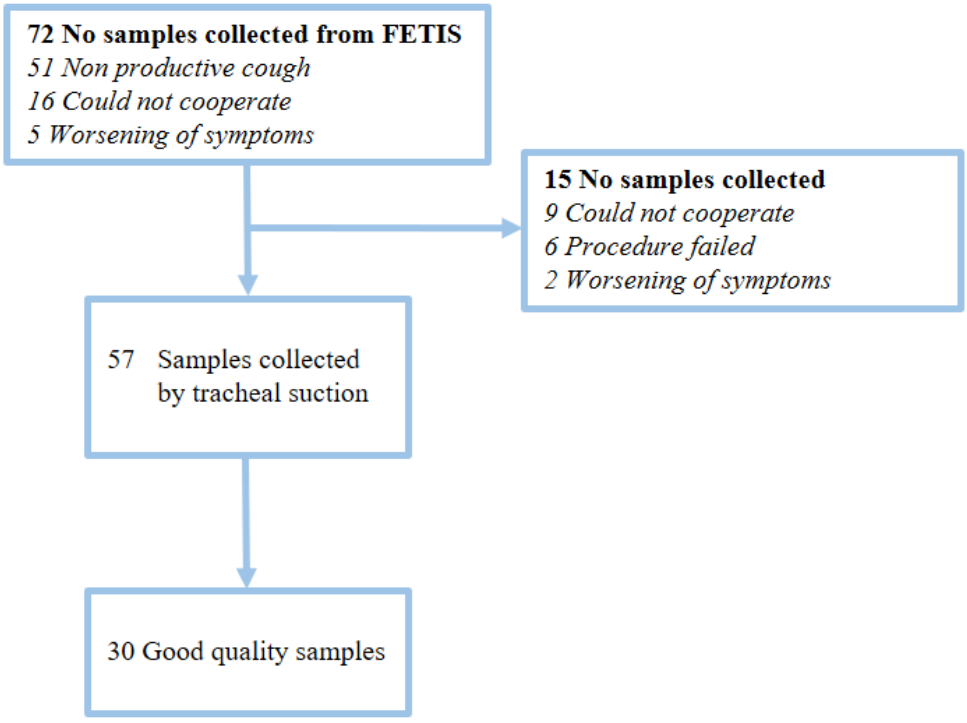


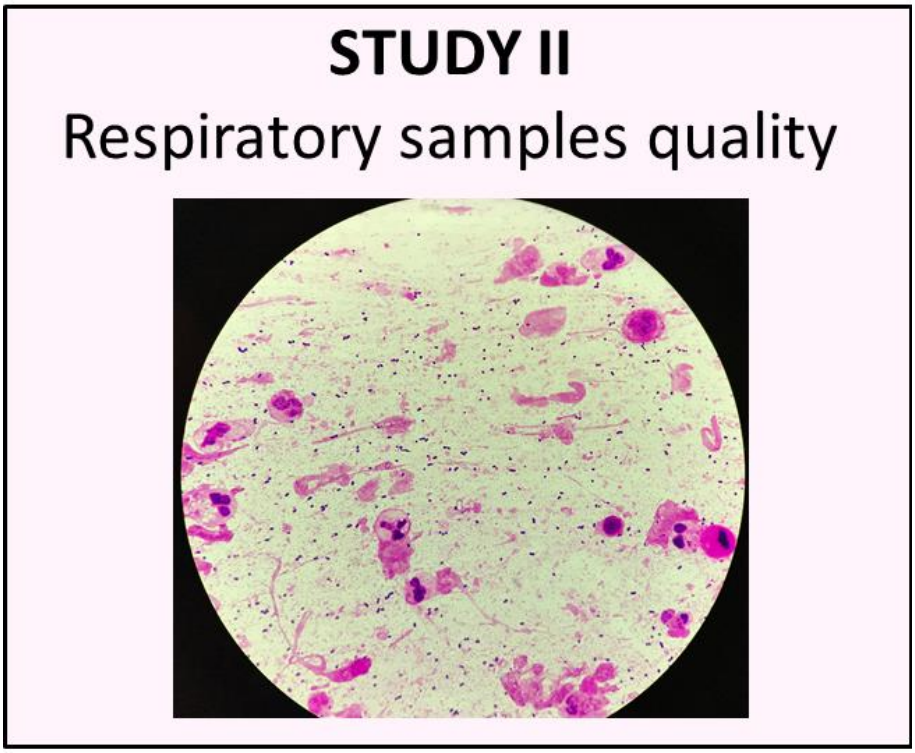
***Figure S7**: Profile of the population with no expectorated samples*

Adverse events	Event, n (%)
Procedure-related bleedings	4 (7%)
Bronchospasms (interrupted procedure)	4 (7%)
Aggravation of dyspnea	2 (3%)

***Table S8:** Harms reported from 57 TS from patients that were unable to expectorate from FETIS group*

As described in the harm section above, one critically ill patient from FETIS group unable to expectorate experienced several adverse events after a tracheal suction procedure (bleeding, dyspnea, oxygen desaturation) where it was necessary to consult a senior physician. All others reported adverse events were considered as mild without the need for further treatment.

11.2.2 Paper II – CONSORT Checklist



CONSORT 2010 checklist of information to include when reporting a randomised trial*

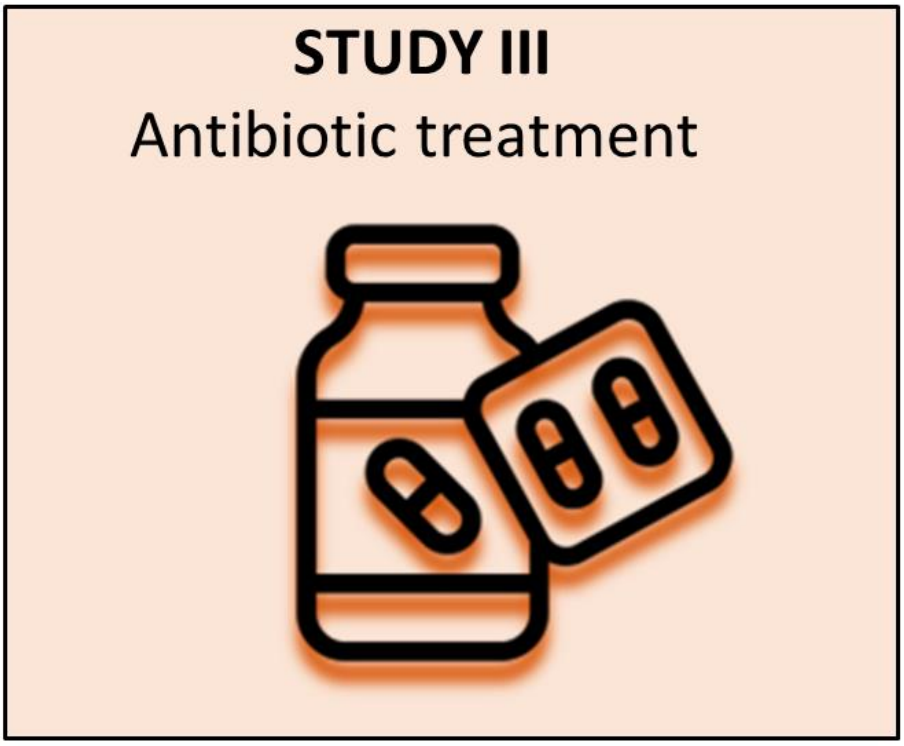
Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	1-2
	2b	Specific objectives or hypotheses	2
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	2
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	3
Participants	4a	Eligibility criteria for participants	2
	4b	Settings and locations where the data were collected	2
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	3
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	3-4

	6b	Any changes to trial outcomes after the trial commenced, with reasons	n/a
Sample size	7a	How sample size was determined	4
	7b	When applicable, explanation of any interim analyses and stopping guidelines	n/a
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	2
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	2
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	2
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	2
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	3
	11b	If relevant, description of the similarity of interventions	n/a
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	4
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	4
Results			
Participant flow (a diagram is	13a	For each group, the numbers of participants who were randomly assigned, received	4 and figure 2

strongly recommended)		intended treatment, and were analysed for the primary outcome	
	13b	For each group, losses and exclusions after randomisation, together with reasons	4 and figure 2
Recruitment	14a	Dates defining the periods of recruitment and follow-up	4
	14b	Why the trial ended or was stopped	n/a
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	5 and 6 (table 1)
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	4 figure 2
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	6 and table 2
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	6 and 7
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	6-7 and supplemental material
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	6-7 and supplemental material
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	9

Generalisability	21	Generalisability (external validity, applicability) of the trial findings	9
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	9
Other information			
Registration	23	Registration number and name of trial registry	2
Protocol	24	Where the full trial protocol can be accessed, if available	2
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	9

11.3 Paper III



The effect of point-of-care multiplex polymerase chain reaction of respiratory specimens on antibiotic treatment of patients acutely admitted with suspected community-acquired pneumonia in Denmark: A multicentre randomised controlled trial

Short title: Antibiotics for pneumonia and the effect of point-of-care testing

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Abstract

Background: Rapid and accurate detection of pathogens is needed in community-acquired pneumonia (CAP) to enable appropriate antibiotics and to slow the development of antibiotic resistance. We aimed to compare the effect of point-of-care (POC) polymerase chain reaction (PCR) detection of respiratory pathogens added to standard care with standard care only (SCO) on antibiotic prescriptions after acute hospital admission.

Methods and Findings: We performed a superiority, parallel-group, open-label, multicenter, randomised controlled trial in three Danish medical emergency departments (EDs) from March 2021 to February 2022. Adults acutely admitted with suspected CAP during the daytime on weekdays were included and randomly assigned (1:1) to POC-PCR (The Biofire® FilmArray® Pneumonia Panel plus added to standard care) or SCO (routine culture and, if requested by the attending physician, target-specific PCR) analysis of respiratory samples. We randomly assigned 294 patients with successfully collected samples (tracheal secretion 78.4% or expectorated sputum 21.6%) to POC-PCR (n=148, 50.4%) or SCO (146, 49.6%). Patients and investigators owning the data were blinded to the allocation and test results. Outcome adjudicators and clinical staff at the ED were not blinded to allocation and test results but were together with the statistician, blinded to data management and analysis. Laboratory staff performing standard care analyses was blinded to allocation. The study coordinator was not blinded. Intention-to-treat and per protocol analysis were performed using logistic regression with clustered standard errors for the prescription of antibiotic treatment. Loss to follow-up comprises three patients in the POC-PCR (2%) and none in the SCO group. Intention-to-treat analysis showed no difference

in the primary outcome of prescriptions of no or narrow-spectrum antibiotics at 4 hours after admission for the POC-PCR (n=91, 62.8%) (OR 1.13 [95% CI, 0.96 to 1.34]; p=0.134) and SCO (n=87, 59.6%). Secondary outcomes showed that prescriptions were significantly more targeted at 4-hours (OR 5.68 [95% CI, 2.49 to 12.94; p=0.000) and 48-hours (OR 4.20 [95% CI, 1.87 to 9.40; p=0.000) and more adequate at 48-hours (OR 2.11 [95% CI, 1.23 to 3.61; p=0.006) and on day 5 in the POC-PCR group (OR 1.40 [95% CI, 1.18 to 1.66; p=0.000). There was no difference between the groups in relation to intensive care unit admissions (OR 0.54 [95% CI, 0.10 to 2.91; p=0.475), re-admission within 30 days (OR 0.90 [95% CI, 0.43 to 1.86; p=0.787), length of stay (IRR 0.82 [95% CI, 0.63 to 1.07; p=0.164), 30-days mortality (OR 1.24 [95% CI, 0.32 to 4.82; p=0.749), and in-hospital mortality (OR 0.98 [95% CI, 0.19 to 5.06; p=0.986).

Conclusion: In a setting with an already restrictive use of antibiotics, adding POC-PCR to the diagnostic set-up did not increase the number of patients treated with narrow-spectrum or without antibiotics. POC-PCR may result in a more targeted and adequate use of antibiotics. A significant study limitation was the concurrent COVID-19 pandemic resulting in an unusually low transmission of respiratory virus.

AUTHORS SUMMARY

Why Was This Study Done?

The global rise in antimicrobial resistance fueled by the excessive use and misuse of antibiotics is a major public health concern.

Fast and accurate diagnostics is important to counteract this development as it can potentially reduce the use of antibiotics/broad-spectrum antibiotics without sacrificing patient safety.

Pneumonia is a common, serious condition where available point-of-care (POC) technology (polymerase chain reaction) allows clinicians to detect possible airway pathogens before treatment decisions are made.

What Did the Researchers Do and Find?

In this randomized trial of 294 patients admitted with suspected pneumonia, POC did not result in the prescription of less antibiotics or less broad-spectrum antibiotics within four hours after admission.

Based on a subset of patients, the results indicated that more patients in the POC-group were treated with targeted or appropriate antibiotics 48 hours and five days after admission.

Patients in the POC-group had a non-statistically significant reduction in length of hospital stay of approximately one day.

What Do These Findings Mean?

The use of respiratory POC does not seem to be an effective tool for reducing the use of antibiotics in a setting with a very low level of antimicrobial resistance and already prudent use of antibiotics.

The use of respiratory POC may aid to ensure a targeted and/or appropriate treatment in a setting with a restrictive use of antibiotics – and thereby may aid to sustain a restrictive strategy.

The concurrent COVID-19 pandemic and the unusually low transmission of common respiratory viruses in the period may have affected the results.

Introduction

Community-acquired pneumonia (CAP) is a leading cause of hospitalisation and mortality [1, 2]. Antibiotic treatment should be initiated timely [3] to avoid serious complications such as bacteremia, sepsis, organ failure, and death [4]. Initial antimicrobial treatment is often empiric, and an uncertain or delayed diagnosis often leads to use of broad-spectrum antibiotics [5]. This, in turn, contributes to adverse effects and complications, such as *Clostridioides difficile* infection, super-infections with resistant bacteria, poor patient outcomes, and general development of antibiotic resistance [6-9]. In Denmark antimicrobial resistance is low, and almost all *S.pneumoniae* are susceptible to benzylpenicillin and 93% to erythromycin, and 75% of *H.influenzae* are susceptible to benzylpenicillin [10]. Danish guidelines recommend narrow-spectrum penicillin for empirical treatment of CAP with CURB-65 < 3 and broad-spectrum antibiotics for severe CAP with CURB-65 ≥ 3. [11, 12]. The CAP diagnosis is based on clinical symptoms such as cough, dyspnea, fever, and sputum production, combined with unspecific diagnostic tools such as auscultation of the lungs, chest radiography, blood tests and microbiological analysis of sputum samples [13-15].

Sputum samples can be cultivated to determine bacterial agents, however, samples are often of poor quality, many patients cannot deliver a sample and laboratory turnaround time is typically 2 days [16, 17]. The lack of precise, timely microbiological results may delay or hinder targeted antimicrobial treatment.

In addition, CAP is often caused by viral infections that can be treated without antibiotics but usually are indistinguishable from bacterial infections without specific microbiological tests [18-20]. Consequently, molecular diagnostic methods, including rapid polymerase chain reaction (PCR) panels for viruses and bacteria, have been developed and tested in clinical settings [21-23]. These panels are simple to use, sensitive, generate rapid results and significantly contribute to the management of CAP [21, 23, 24].

By identifying pathogenic organisms earlier, studies have reported faster de-escalation of antibiotic treatment, reduced duration of broad-spectrum empirical antibiotic therapy, reduced length of stay (LOS), and reduced hospital costs [25, 26]. However, evidence of clinical impact of point-of-care (POC)-PCR testing of sputum samples in EDs is limited and a recent feasibility study advocates the need for randomised controlled trials (RCT) to test POC-PCR panels in acute settings [27].

In this multicenter, randomised study, we aimed to investigate the effect of adding POC-PCR to standard care in an ED setting. Our hypothesis was that POC-PCR testing of sputum samples from suspected CAP patients would increase the proportion of patients treated with no or narrow-spectrum antibiotics. The objectives were i) To investigate the effect of POC-PCR testing of sputum from suspected CAP patients on the prescriptions of antibiotic treatment compared to usual care and ii) To investigate if the addition of POC-PCR testing to the diagnostic set-up affects length of stay (LOS), intensive care unit (ICU) admission, 30-days mortality, in-hospital mortality or re-admissions within 30 days.

Methods

Trial design

This study was designed as a superiority, parallel-armed, multicenter randomised controlled clinical trial, and was part of a large multifaceted clinical study "INfectious Diseases in EmErgency Department" (INDEED) [28].

The study was reported in accordance with the Consolidation Standard of Reporting Trials (CONSORT) guidelines [29]. The processing of personal data is notified to and approved by the Region of Southern Denmark and listed in the internal record (no. 20/60508) cf. Art 30 of The EU General Data Protection Regulation, and approved by the Regional Committee on Health Research Ethics for Southern Denmark (S-20200188), registered by ClinicalTrials.gov (NCT04651712), and conducted according to the Declaration of Helsinki-Ethical principle for medical research involving human subjects. The study protocol has been published and includes further information about the methods [28].

Setting

The trial was conducted in three Danish medical EDs with a coverage of approximately 750.000 inhabitants: two regional hospitals, Lillebælt Hospital in Kolding and Hospital Sønderjylland in Aabenraa, and one university hospital, Odense University Hospital in Odense. Based on data from the National Health Data Agency and Statistics Denmark, the mean hospital LOS for patients > 65 years old hospitalised in departments with medical specialties (including pneumonia) was of 5.9 days in 2018 [30], and local data from the three hospitals included in this study, reported a mean LOS of 3.8

days in hospital for adult patients (>18 years) discharged with pneumonia diagnose during the study period. According to clinical guidelines, patients admitted to the ED in our institutions must have a clinical assessment within half an hour to clarify suspicion of infection and disease severity. If the ED physician suspects CAP, diagnostic biomarkers, Chest X-ray, and tracheal suctioning/aspirates, or expectorated sputum are performed without delay [11, 12]. If indicated, empirical treatment must be initiated within 4-hours, and the treatment must be documented in the patient medical chart. The empirical treatment for CAP is presented in Table S1, and the timeline for the standard procedures in the EDs is presented in Table S2.

Participants

Adults aged 18 years or older admitted to the ED were invited to participate in the study if the attending physician suspected CAP and the patient had at least one of the following pulmonary symptoms: dyspnea, cough, expectoration, chest pain, or fever. Patients were excluded if: they could not deliver a sputum sample, participation delayed urgent treatment, the patient was transferred to an intensive care unit, the patient had been admitted within the last 14 days, had COVID-19 infection at admission, was pregnant, or had severe immunodeficiencies (HIV positive, with a cluster of differentiation 4 cell count <200), treatment with immunosuppressive medicine (Anatomical Therapeutic Chemical classification L04A), corticosteroids (>20 mg/day prednisone or equivalent for >14 days within the last 30 days) or chemotherapy within 30 days [28]. If patients fulfilled the eligibility criteria, the study assistant obtained verbal and written consent at the bedside right after the clinical assessment and before inclusion in the study. Patients were recruited consecutively Monday through Friday from 10 a.m. to 8 p.m.

Randomisation and masking

The patient was randomly assigned to one of two groups with 1:1 allocation: 1) POC- PCR analysis (Biofire FilmArray Pneumonia Panel plus, Biomérieux, Marcy l'Etoile, France) [31] in addition to standard care, or 2) standard care only (SCO) as control. The randomisation was generated electronically using Research Electronic Data Capture Randomisation Module [32]. Computer-generated random lists were prepared by an independent data manager with permuting blocks of varying size and stratified according to sites. Allocation concealment was ensured, as randomisation was performed electronically, and the study assistants administering the randomisation did not have access to the randomisation code. The allocation was not revealed to the project assistant before consent was obtained and specimen collected. Patients and investigators owning the data were blinded to the allocation and test results. Outcome adjudicators and clinical staff at the ED were not blinded to allocation and test results but were together with the statistician, blinded to data management and analysis. Laboratory staff performing standard care analyses was blinded to allocation. The study coordinator was not blinded.

Procedure

Tracheal secretion is the recommended sampling method by Danish national and regional guidelines [11, 12], but expectorated sputum is accepted if the patient can not cooperate during the procedure. LRT specimens were collected right after enrolment by a project assistant. Tracheal suction/aspiration was performed with a catheter (EXTRUDAN Surgery Aps, Denmark, CH12, 530 mm) insertion into the nares during inhalation. The catheter was gently advanced about 40 cm into the trachea, where suctioning

at 200–400 mmHg was performed before withdrawing the catheter. POC-PCR analysis was done without delay in a POC laboratory. The POC laboratory had 24-hour coverage and was situated in the ED (two sites) or close to the department (transport time less than 10 minutes, one site). Project assistants and laboratory staff were trained in the use of the POC-PCR system, and each site had a pocket laboratory protocol to ensure sample quality and safe handling of specimens. Within 4-hours after the patient was admitted, the result of the POC-PCR was handed to the treating physician along with a guideline-based action card (see Text S3) recommending specific treatments matching different POC-PCR results. In case of any additional questions, the physician was encouraged to contact the local clinical microbiologist for further advice. All six project assistants received bedside training in tracheal suction to ensure consistent data collection. Clinical and patient data were retrieved by chart review and patient interview as described in the protocol [28].

Intervention

Point-of-care polymerase chain reaction (POC-PCR)

The Biofire® FilmArray® Pneumonia Panel plus (Biomérieux, Marcy l'Etoile, France) is an automatic, closed, multiplex PCR, that includes all steps of molecular diagnostics in about 75 min, including sample preparation. The panel detects 18 bacterial pathogens, 9 viruses and 7 antimicrobial resistance genes (see Table S4).

Results for typical colonizing bacteria were reported semi-quantitatively providing estimates to the nearest whole log as gene copies/ml ranging from

10^4 to 10^7 copies/ml. Biofire® FilmArray® Pneumonia Panel was used in accordance with the manufacturer's instructions at all three sites. [31]. All POC-PCR results were registered directly in a study database and in the patient's medical chart.

Standard care (Routine culture and PCR)

All samples were submitted to standard-of-care procedures of microbiological testing. Part of the sputum sample was transferred to a 5% blood agar plate and to a chromogenic and/or selective agar. The inoculum was streaked over the agar surface and blood agar plates were inoculated with a *Staphylococcus* streak to allow growth of *Haemophilus influenzae*. Blood agar plates were incubated in a 5% CO2 atmosphere, other plates at 35 º C in normal atmospheric conditions. After 1-2 days of incubation, pathogens were identified by Matrix-Assisted Laser Desorption/Ionization-time of flight, and reported semi-quantitatively as few, some or numerous. In addition, "no growth of pathogens" and "upper airway microbiota" were reported. Routine PCR was performed if requested by the referring physician (e.g. for *Legionella pneumophila* or influenza virus).The results were registered in the microbiological laboratory information system (MADS, Aarhus University Hospital, Aarhus, Denmark) and were accessible from the patient's medical chart.

Outcomes

The primary outcome was the prescription of "no or narrow-spectrum" antibiotics within four hours after admission. Narrow-spectrum antibiotics were defined as antibiotics active against CAP pathogens: Beta-lactamase sensitive penicillins (phenoxymethylpenicillin or benzylpenicillin), extended spectrum beta-lactamase sensitive penicillins (ampicillin/amoxicillin/pivampicillin). In case of penicillin allergy: macrolides and cefuroxime were also defined as narrow-spectrum antibiotics (See Table S5). We pooled narrow-spectrum and no antibiotics, as our focus was rational and restrictive use of antibiotics [11, 12]. As our main focus was to study POC-PCR from an antibiotic stewardship perspective, we decided to handle no and narrow-spectrum antibiotics as our primary outcome and targeted antibiotics as a secondary outcome. In the initial protocol, no, narrow-spectrum, and targeted antibiotics were treated as a composite primary outcome [28].

Secondary outcomes:

Prescription of no or narrow-spectrum antibiotics at 48 hours and 5 days after admission

Prescription of targeted antibiotics within 4 hours, 48 hours and 5 days. Targeted antibiotics were defined as either narrow-spectrum antibiotics targeting CAP or antibiotics directed against a detected bacterial pathogen identified by culture.

Prescription of adequate antibiotics within 4 hours, 48 hours, and 5 days. Adequate antibiotics were defined as all antibiotics covering the detected bacterial pathogen.

We categorized antibiotic treatment as targeted and/or adequate in relation to the following pathogens identified by culture: *Streptococcus pneumoniae, H. influenzae, Moraxella catarrhalis, Pseudomonas aeruginosa, Staphylococcus aureus*, hemolytic streptococci, and *L. pneumophila* (see, Table S6). We excluded *Enterobacterales, Acinetobacter*, and yeast as they usually represent colonization and are less likely to cause CAP.

Data on other secondary outcomes were extracted from the patients' medical chart: 30 days-mortality (death within 30 days from admission to the ED), in-hospital mortality (death during the current hospitalization, ICU admission during the current hospitalization, re-admission within 30-days after discharge and LOS (days from admission to discharge).

Statistical methods

Based on literature and local data, we assumed that adherence to antimicrobial guidelines was 50% for the management of CAP patients [33], and we required at least 200 patients with suspected CAP with two-sided 5% significance to achieve a power of 82% to detect a minimal difference of 20% prescription of no or narrow-spectrum treatment in the POC-PCR group compared to the control group. However, more patients were included, so the power calculation was repeated without changing earlier assumptions before the commencement of statistical analysis and with the statistician blinded to the allocation groups and the general distribution of the data. The new calculation yielded a power of 94% with 290 patients with two-sided 5% significance.

Descriptive statistics were conducted to assess whether the exchangeability assumption was met for the baseline variables. To assess whether there was a difference between the two groups Fisher's exact test or chi-square test were performed for categorical variables, and t-test or Wilcoxon ranksum test for non-categorical variables.

To accommodate the variation between study sites, we used logistic regression with clustered standard errors to investigate the effect of POC-PCR on antibiotic prescription within 4-hours, 48-hours, and 5 days. To compare the two groups, we used negative binomial regression for LOS. Logistic regression analyses were performed for 30 days-mortality, in-hospital mortality, ICU admission, and re-admission within 30 days and unadjusted and adjusted for triage. Multiple imputation was performed to handle missing data. We considered a two-sided p-value less than 0.05 statistically significant, and no adjustments for multiple testing were utilized. Statistical analyses were performed using STATA 17.0 (TX, USA).

Results

Patients admitted with suspected CAP were enrolled from March 1, 2021, to February 28, 2022. The last follow-up for mortality and re-admission was on April 1, 2022. We screened 379 patients for eligibility and collected 294 (77.6%) LRT samples (78.4 % tracheal secretions and 21.6% expectorated sputa) from patients who underwent randomisation. The 294 patients were allocated to either the POC-PCR group (148 patients (50.4%)) or the SCO group (146 patients (49.6%)), and those patients were included in the intention-to-treat analysis. Per protocol analyses for the primary outcome included 291 (99.0%) patients with no or narrow antibiotic treatment

registered within 4 hours (POC-PCR 145 (49.8%) and SCO 146 (50.2%)) (Figure 1).

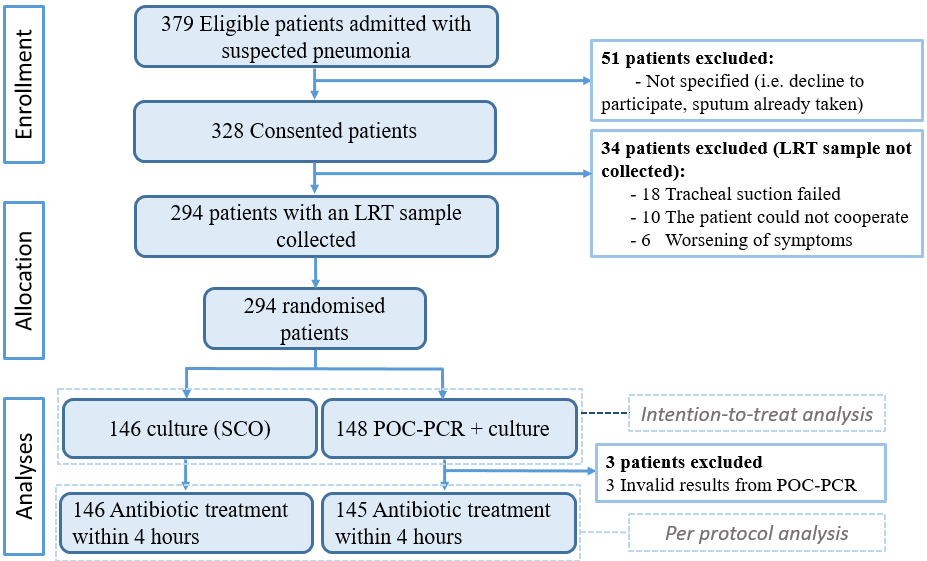

Figure 1: Trial profile
LRT: Lower respiratory tract; SCO: Standard care only; POC-PCR: Point-of-care polymerase chain reaction

Baseline data

Demographic and clinical characteristics are shown in Table 1.

Table 1: baseline characteristics of patients included in the analysis

Allocation	SCO n=146	POC-PCR n=145	Total n=291
Age, median years (IQR)	72.5 (59.0; 81.0)	74.0 (61.5; 81.0)	73.0 (60.0; 81.0)
Gender (male), n (%)	70 (47.9)	78 (53.8)	148 (51.0)
ADL [a], n (%)	45 (31.0)	33 (22.3)	78 (26.6)
Nursing home resident, n (%)	15 (10.3)	14 (9.5)	29 (9.9)
Patients with a confirmed CAP diagnosis [b], n (%)	83 (56.8)	89 (61.4)	172 (59.1)

HRCT findings suggestive of pneumonia, n (%)	66 (45.2)	79 (54.5)	145 (49.8)
Type of respiratory samples, n (%)			
Tracheal secretions	112 (76.7)	116 (80.0)	228 (78.4)
Expectorated sputa	34 (23.3)	29 (20.0)	63 (21.6)
Blood culture, n (%)	127 (86.9)	120 (83.3)	247 (85.2)
Bloodstream infections	12 (8.2)	6 (4.1)	18 (6.2)
Urine culture, n (%)	124 (84.9)	119 (82.6)	243 (83.8)
Bacteriuria [c]	25 (20.2)	34 (28.3)	59 (24.2)
SYMPTOMS			
Cough n (%)	102 (71.3)	104 (72.2)	206 (71.8)
Expectoration, n (%)	85 (59.4)	78 (54.2)	163 (56.8)
Breast tightness, n (%)	44 (31.2)	46 (31.7)	90 (31.5)
Dyspnea, n (%)	104 (72.7)	108 (75.0)	212 (73.9)
SEVERITY ASSESSMENT			
CURB-65 [d] ≥ 3, n (%)	24 (16.4)	18 (12.4)	42 (14.4)
Glasgow Coma Scale < 15, n (%)	7 (4.8)	6 (4.1)	13 (4.4)
Triage [e] ≥ 2, n (%)	62 (42.5)	40 (27.6)	102 (35.1)†
COMORBIDITIES			
Chronic obstructive pulmonary disease, n (%)	42 (28.8)	51 (35.2)	93 (32.0)
Neurological disease, n (%)	27 (18.5)	28 (19.3)	55 (19.0)
Cardiovascular disease, n (%)	56 (38.4)	62 (42.8)	118 (40.5)
Endocrinological disease, n(%)	49 (33.6)	43 (29.7)	92 (31.6)
VITAL PARAMETERS			
Oxygen saturation, median (IQR)	94.0 (91.0; 96.0)	93.0 (92.0; 96.0)	94.0 (92.0; 96.0)
Respiratory frequency/min, median (IQR)	22.0 (20.0; 25.0)	20.0 (18.0; 24.0)	22.0 (18.0; 24.0)
Heart rate/min, mean (sd)	93.8 (18.2)	92.2 (17.6)	93.0 (17.9)
Systolic blood pressure mmHg, mean (sd)	134.7 (20.3)	135.4 (22.1)	135.0 (21.2)
Diastolic blood pressure mmHg, mean (sd)	75.2 (14.5)	76.0 (16.9)	75.6 (15.7)
Temperature ºC, mean (sd)	37.6 (1.0)	37.5 (0.9)	37.6 (1.0)
BLOOD TESTS			
C-reactive protein mg/L, median (IQR)	86.5 (30.8; 170.8)	82.0 (30.5;178.0)	82.0 (31.0; 174.0)

Leucocytes 10^9/L, median (IQR)	11.1 (8.5; 15.6)	11.3 (8.5; 14.8)	11.2(8.5; 15.2)
Neutrophils 10^9/L, median (IQR)	8.2 (6.0; 13.1)	8.9 (6.2; 12.5)	8.7 (6.1; 12.6)
ANTIBIOTIC TREATMENT and VACCINE STATUS			
Antibiotic treatment before admission [f], n (%)	38 (26.0)	36 (24.8)	74 (25.4)
Antibiotic treatment at admission, n (%)	32 (21.9)	30 (20.7)	62 (21.3)
Allergy to antibiotics, n (%)	9 (6.2)	12 (8.3)	21 (7.2)
Pneumococcal vaccine within 5 years, n (%)	75 (51.4)	84 (57.9)	159 (54.6)
Influenza vaccine (season 2020/2021), n (%)	103 (70.5)	(72.4)	208 (71.5)

Data are n (%): numbers (percentages), median (IQR: Interquartile range), or mean (SD: Standard deviation).

[a] Activities of daily living: One or more dependencies related to bathing, dressing, toileting, transfer, continence, and eating; [b] The confirmed CAP (Community-acquired pneumonia) diagnosis was assigned by an expert panel of experienced emergency- and infectious disease experts in acute infections based on all clinical information from the medical record within the first week of ED admission, including a chest computed tomography; [c] Bacteriuria >10^4 bacteria/mL (E*nterobacteriaceae)* or >10^5 (others);[d] CURB-65: confusion, blood Urea nitrogen >7 mmol/l, Respiratory rate ≥30 breaths per minute, Blood pressure <90 mmHg systolic or ≤60 mmHg diastolic, age ≥65 years; [e] Triage: Danish emergency process triage [34]; [f] Antibiotic treatment within one month prior to admission;† p=0.001; SOC: Standard care only; POC-PCR: Point-of-care Polymerase chain reaction; CAP: Community-acquired pneumonia; HRCT: High resolution computed tomography; mmHg: millimetre(s) of mercury; mg/L: milligrams per litre

Number of patients prescribed “no or narrow”, targeted, and adequate antibiotic at 4-hours, 48hours, and 5 days is presented in Table 2. Because of the observed difference in triage between the intervention and control, unadjusted and adjusted results are presented in Tables 3 and 4.

Table 2: Absolute values for "no or narrow (no and narrow), targeted and adequate treatments" at 4-hours, 48-hours, and day 5. Analyses of targeted and adequate treatment were based on 55 positive culture results from 290 patients.

Patients with prescriptions of "no or narrow" antibiotic treatment (AT)									
Time-line	4-hours, n=291 (%)			48-hours, n=291 (%)			5th Day, n=290 (%)		
	POC-PCR[1]	SCO[2]	Total	POC-PCR[1]	SCO[2]	Total	POC-PCR[1]	SCO[2]	Total
	145 (49.8)	146 (50.2)	291 (100)	145 (49.8)	146 (50.2)	291 (100)	144 (49.7)	146 (50.3)	290 (99.7)
No or Narrow AT	91 (62.8)	87 (59.6)	178 (61.2)	88 (60.7)	90 (61.6)	178 (61.2)	88 (61.1)	95 (65.1)	183 (63.1)
No AT	30 (20.7)	29 (19.9)	59 (20.3)	31 (21.4)	28 (19.2)	59 (20.3)	33 (22.9)	36 (24.7)	69 (23.8)
Narrow AT	61 (42.1)	58 (39.7)	119 (40.9)	57 (39.3)	62 (42.4)	119 (40.9)	55 (38.2)	59 (40.4)	114 (39.3)
Patients with positive culture results									
Time-line	4-hours, n=55 (%)			48-hours, n=55 (%)			5th Day, n=55 (%)		
	POC-PCR[1]	SCO[2]	Total	POC-PCR[1]	SCO[2]	Total	POC-PCR[1]	SCO[2]	Total
	26 (47)	29 (53)	55 (100)	26 (47)	29 (53)	55 (100)	26 (47)	29 (53)	55 (100)
Target AT	15 (57.7)	7 (24.1)	22 (40.0)	17 (65.4)	10 (34.5)	27 (49.1)	14 (53.9)	15 (51.7)	29 (52.7)
Adequate AT	19 (73.1)	17 (58.6)	36 (65.5)	20 (76.9)	18 (62.1)	38 (69.1)	19 (73.1)	19 (65.5)	38 (69.1)

[1]Point-of-care polymerase chain reaction in addition to routine culture [2]Standard care only

Prescription of no or narrow-spectrum antibiotics

There were three missing samples due to POC-PCR assay failure. Thus, no clinical characteristics influenced the missing mechanism. Therefore, we believe the data are missing completely at random. However, for sensitivity reasons, multiple imputation was performed. Results from per protocol and intention-to-treat analysis were similar. POC-PCR was not superior to SCO regarding prescriptions of no or narrow-spectrum antibiotics within 4-hours after admission. Intention-to-treat analyses of 294 patients yielded an OR of 1.13 [95% CI, 0.96 to 1.34]; p=0.134, and per protocol analysis of 291 patients resulted in an OR of 1.14 [95% CI, 0.97 to 1.34]; p=0.101. We found a statistically significant difference on day 5 but not 48-hours after admission (Table 3).

Prescription of targeted and adequate antibiotics

Pre-specified analysis of targeted antibiotic treatment and exploratory analyses of adequate antibiotics were based on positive culture results from 290 specimens after exclusion of one sample missing from the culture analysis. We identified 68 (23%) bacterial agents from 55 (19%) patients. Targeted treatment was used significantly more often in the POC-PCR compared with the SCO group at both 4 hours and 48 hours but not at day 5 (Table 3). Analysis of adequate treatment did not show a statistically significant difference between the groups at 4-hours but more patients were treated with adequate antibiotics at 48-hours and on day 5 in the POC-PCR compared to the SCO group (Table 3). A graphical presentation of changes in (a) no or narrow, (b) targeted and (c) adequate treatment for both groups is presented in Figure 2.

Table 3: Unadjusted and adjusted per protocol analyses for the primary and secondary outcomes: prescriptions of no or narrow, targeted, and adequate antibiotic treatment (AT) at 4-hours, 48-hours, and day 5. The control group (SCO;standard care only)) is the reference. Analyses of targeted and adequate treatment were based on 55 positive culture results and routine PCR from 290 patients.

Timeline	**4-hours (n=291)**		**48-hours (n=291)**		**5 days (n=290)**	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Primary outcome						
No or narrow AT	1.14 (0.97;1.34)	0.101	-	-	-	-
Adjusted for triage	1.05 (0.73;1.51)	0.772				
Secondary outcomes						
No or narrow AT	-	-	0.96 (0.87;1.04)	0.373	0.84 (0.73;0.97)	0.021
Adjusted for triage	-	-	0.91 (0.82;1.00)	0.065	0.81 (0.72;0.91)	0.001
Timeline	**4-hours (n=55)**		**48-hours (n=55)**		**5 days (n=55)**	
Secondary outcomes						
Target AT	4.28 (2.51;7.32)	0.000	3.58 (1.39;9.26)	0.008	1.09 (0.65;1.83)	0.749
Adjusted for triage	5.68 (2.49;12.94)	0.000	4.20 (1.87;9.40)	0.000	1.08 (0.61;1.91)	0.786
Adequate AT	1.91 (0.68;5.40)	0.219	2.04 (1.32;3.14)	0.001	1.43 (1.33;1.54)	0.000
Adjusted for triage	2.11 (0.56;7.96)	0.267	2.11 (1.23;3.61)	0.006	1.40 (1.18;1.66)	0.000

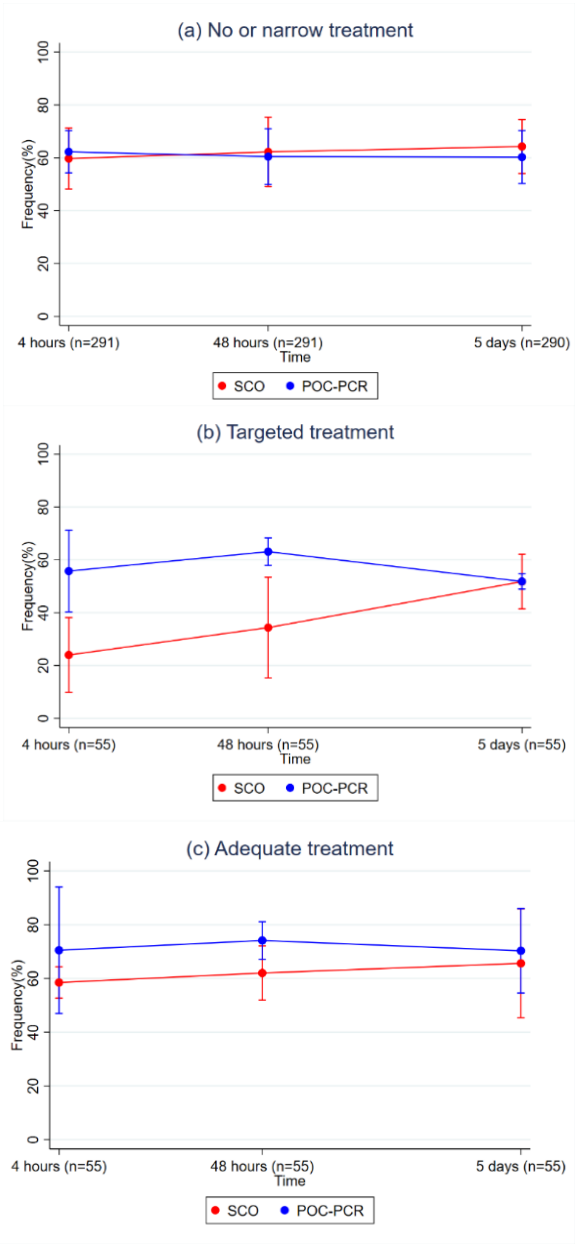

Figure 2: Changes in (a) no or narrow-, (b) targeted-, and (c) adequate treatment prescription at 4-hours, 48-hours, and day 5. Targeted and adequate treatments are based on culture results and routine PCR and include a sample of 55 patients. Results were adjusted for triage. SCO: Standard care only; POC-PCR: Point-of-care polymerase chain reaction

Adverse events

There were no statistically significant differences between POC-PCR and SCO regarding patient 30-day mortality, in-hospital mortality, admission to intensive care unit, 30-day re-admission, and LOS (table 4).

Table 4: Adverse events and length of stay for 291 patients

Adverse Events	SCO Event (n=146)	POC-PCR Event (n=145)	OR (95% CI) p-value Crude	OR (95% CI) p-value Adj. for triage
30-days mortality[1]	4	5	1.26 (0.33;4.81) 0.728	1.24 (0.32;4.82) 0.749
In-hospital mortality[2]	3	3	1.00 (0.19;5.07) 0.993	0.98 (0.19;5.06) 0.986
Admission to ICU[3]	5	2	0.39 (0.07;2.06) 0.271	0.54 (0.10;2.91) 0.475
Re-admission to hospital[4]	20	17	0.83 (0.41;1.67) 0.614	0.90 (0.43;1.86) 0.787
Adverse events in total[5]	32	27	0.96 (0.51;1.77) 0.896	1.04 (0.55;1.97) 0.899
	Days	Days	IRR (95% CI), p-value	
LOS[6] (average in days)	5.2	4.2	0.80 (0.62;1.04), 0.098	
Adjusted for triage	4.3	3.6	0.82 (0.63;1.07), 0.164	

[1]Mortality within 30 days from admission to the Emergency Department [2]Patient mortality during the current hospitalization [3]Transfer to intensive care unit during the current hospitalization [4]Admission within a 30-day period after discharge from current admission [5]Total of numbers of adverse events per patient. [6]Defined as the time (in days) spent in hospital during the current admission (days from admission to hospital discharge).

Discussion

In this randomised study, adding sputum-POC-PCR to our diagnostic set-up did not affect prescriptions of no or narrow-spectrum antibiotics during the first two days of admission, but less patients in the POC-PCR-group were treated with no or narrow-spectrum antibiotics after five days. Interestingly, patients in the POC-PCR-group were more likely to receive early targeted and adequate treatment. Number of re-admissions, ICU admissions and mortality were unchanged but we found a non-significant one-day reduction in LOS.Several prospective studies have reported sputum-POC-PCR as a method to support clinical decisions by fast and accurate detection of CAP pathogens [21-23, 35]. Studies have shown a reduction in both use of intravenous antibiotics and number of days treated with antibiotics. In contrast to our study, most previous studies in ED-settings only used panels for detecting upper respiratory pathogens [25, 26, 36, 37].

Our outcomes were different focusing on type of antibiotic instead of length of treatment and route of administration, but nevertheless, the failure of POC-PCR to increase the use of no or narrow-spectrum antibiotics may seem to contrast these previous results.

There are some likely explanations. In an international context, the level of antimicrobial resistance is very low in Denmark and most pneumococci and *H. influenzae* are susceptible to penicillins [10]. Consequently, Danish treatment guidelines recommend relatively narrow-spectrum penicillins for CAP and reserve broad-spectrum antibiotics for severe pneumonia and/or sepsis [11, 12]. This may have affected the study. For instance, a patient with severe CAP may have been treated with penicillin instead of broad-spectrum antibiotics if POC-PCR detected pneumococci and another patient with mild

CAP may have been treated with broad-spectrum antibiotics instead of penicillin if POC-PCR detected *M. catarrhalis*. Both actions were in agreement with the provided action card and both actions would result in a more targeted treatment - but also blur the effect of POC-PCR. This explanation is in line with the observation that patients in the POC-PCR group were more likely to receive early targeted and adequate treatment. In addition, the detection of *Enterobacterales* and *Pseudomonas aeruginosa* with POC-PCR may result in broad antimicrobial therapy even though they rarely cause CAP in a medical ED [38]. We excluded *Enterobacterales* from the analysis of targeted and adequate treatment due to the low incidence (1.3%) and because they usually represent colonisation [38].

Another possible explanation is the very low prevalence of common respiratory viruses in the study period related to the SARS-CoV-2 pandemic [39]. In other studies, virus accounted for 20-40 % of CAP cases [19, 20, 37]. Some patients with CAP and a detected viral cause may be treated without antibiotics, and it is therefore possible that POC-PCR would have reduced the use of antibiotics in a period with a higher transmission of respiratory viruses.

The increased prescription of targeted and adequate antibiotics in the POC-PCR-group within the first two days is an interesting observation. It is based on analysis of a small subset of culture-positive samples, therefore it is unknown if the result completely or in part can be extrapolated to the rest of the study population. Nevertheless, it highlights the question if POC-PCR improves patient outcome. We did not find any difference in mortality or transferal to ICU - but the number of events was very low. There was no difference in the number of re-admissions but we did find a non-significant reduction in LOS from 4.3 to 3.6 days (p=0.164) when adjusted for triage. It

was not significant, but it might on the other hand reflect improved patient treatment and a possible reduction in LOS of almost 20% for one of the most common infections in the ED is very interesting from a hospital management and economic perspective.

At day five, more patients in the SCO-group were treated with no or narrow-spectrum antibiotics and there was no difference in the use of targeted antibiotics. This observation may be explained by routine microbiological results being available between day two and five – allowing adjustment of treatment. Even though, we detected statistically significant differences they might be without clinical significance as they were quite small and day 5 is at the end of our recommended treatment duration.The strength of our study is the pragmatic multicentre, randomized controlled trial design. The randomised design ensured that severity of illness, CAP diagnosis, and other patient characteristics were distributed equally between intervention and control group, and therefore causal inference is likely as the assumption of positivity is fulfilled. The POC-PCR analysis was integrated in the usual workflow in our ED suggesting that the test is technically feasible and easy to implement in other EDs. Project assistants were trained in collecting LRT-specimens and in using the POC-PCR-platform and the primary investigator monitored the project closely to ensure a high level of internal validity. Almost 80% of the collected samples were tracheal secretions and this may have increased the reliability of the microbiological results by reducing upper airway contamination [40, 41]. To ensure a uniform and correct clinical interpretation we provided all POC-PCR results with a clear guideline-based action card.

There are also a number of limitations. Only few patients with CURB-65 scores ≥ 3 (14.4%) were included in the study. The inability to consent is likely linked to severe disease and acute cognitive impairment. In addition, restriction to weekdays and daytime may have reduced the number of severe cases as admission on weekends and at night are known to be associated with increased mortality and risk of referral to ICU [42]. Therefore results can only be generalised to patients admitted on weekdays during daytime. In the secondary analysis of targeted and adequate treatment, only few culture-positive samples were included. The sensitivity of culture may be very low, and a high number of patients were treated with antibiotics before admission [43]. We could have circumvented this challenge by also analysing samples in the SCOgroup with FilmArray® with a random disclosure design where results only are available in the intervention group. It would also allow subgroup analysis to investigate the effect of POC-PCR separately in test-positive and test-negative patients. It would straighten the results, leading to evidence-practice recommendations for implementing the test in clinical practice. However, it would be more expensive and may introduce ethical issues [44, 45].

Both culture and POC-PCR may detect commensals, which was stated clearly in the provided action card. It is therefore possible that the clinicians in some situations chose to ignore the result - e.g. based on severity of illness, response to current treatment, fear of prescribing inadequate treatment, likelihood of commensal pathogen and expected virulence of the pathogen [46]. We did not measure to what extent the action card recommendations were followed.

A possible interpretation of the overall results is that the current restrictive prescribing strategy in Denmark may be unable to provide targeted and

adequate treatment for some patients. This may be overcome by introducing broad-spectrum empirical regimes - but that would fuel a further rise in resistance, may introduce side effects, and go against our general antimicrobial stewardship interventions. However, as indicated in this study, we might get around this problem by introducing fast and sensitive diagnostic methods. Future studies should focus on 1) the impact of POC-PCR on clinical outcome in a larger scale - e.g. LOS, length of treatment and patient quality of life, 2) hospitalisation costs and 3) the use of adequate and target treatment in a blinded setup where sensitive molecular methods are applied in both intervention and control groups.In conclusion, in this randomised trial introduction of POC-PCR did not increase the proportion of patients prescribed no or narrow-spectrum antibiotics but it might increase early treatment with adequate and targeted antibiotics and may be associated with a reduced LOS. The results apply to a setting with restrictive use of antibiotics and a very low level of antimicrobial resistance and may be quite different in other settings. Fast and accurate diagnostic tools may aid to maintain a restrictive use of antibiotics in the future.

Supporting Information

Table S1: Empirical treatment guidelines of CAP of the region of Southern Denmark

Table S2: Standard care procedures in our institutions

Text S3: Action card

Table S4: Targets of the Biofire® FilmArray® Pneumonia Panel plus

Table S5: Classification of ”Narrow antibiotic” treatment

Table S6: Classification of ”targeted and adequate” treatment

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Authors' contributions: MBC, FSR, CBM, TS, SLA, AKP and, HSA were involved in the study's conceptualization and design. MBC performed the literature search and drafted the original work in collaboration with HSA. MBC coordinated the project and participated in recruitment, data supervision, monitoring, and collection. AKP supervised the statistical analyses. HSA was the chief research officer responsible for supervising the overall study and participated in data management. All authors, HSA, FSR, CBM, TS, SLA, and AKP, critically revised and approved the final manuscript. HSA and CBM were responsible for the overall content as guarantors.

Abbreviations

CAP, community-acquired pneumonia; CI, confidence interval; CONSORT, consolidation standard of reporting trials; CURB-65, confusion, blood Urea nitrogen >7 mmol/l, Respiratory rate ≥30 breaths per minute, Blood pressure <90 mmHg systolic or ≤60 mmHg diastolic, age ≥65 years; ED, emergency department; HVI, human immunodeficiency virus; ICU, intensive care unit; IRR, incidence rate ratio; LOS, length of stay; LRT, lower respiratory tract; LRTI, lower respiratory tract infection; OR, odds ratio; PCR, polymerase chain reaction; POC, point-of-care; SCO, standard care only

References:

1. Søgaard M, Nielsen RB, Schønheyder HC, Nørgaard M, Thomsen RW. Nationwide trends in pneumonia hospitalization rates and mortality, Denmark 1997-2011. Respir Med. 2014;108(8):1214-22.doi:10.1016/j.rmed.2014.05.004

2. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Infect Dis. 2018;18(11):1191-210.doi:10.1016/s1473-3099(18)30310-4

3. National Institute for Health and Care Excellence. Pneumonia in Adults: Diagnosis and Management – NICE Guideline. London: Royal College of Physicians.: NICE; [accessed on 2020 October 12]. Available from: https://www.rcplondon.ac.uk/guidelines-policy/pneumonia-adults-diagnosis-and-management-nice-guideline.

4. Meehan TP, Fine MJ, Krumholz HM, Scinto JD, Galusha DH, Mockalis JT, et al. Quality of care, process, and outcomes in elderly patients with pneumonia. JAMA. 1997;278(23):2080-4

5. Braykov NP, Morgan DJ, Schweizer ML, Uslan DZ, Kelesidis T, Weisenberg SA, et al. Assessment of empirical antibiotic therapy optimisation in six hospitals: an observational cohort study. Lancet Infect Dis. 2014;14(12):1220-7.doi:10.1016/s1473-3099(14)70952-1

6. Becerra MB, Becerra BJ, Banta JE, Safdar N. Impact of Clostridium difficile infection among pneumonia and urinary tract infection hospitalizations: an analysis of the Nationwide Inpatient Sample. BMC Infect Dis. 2015;15:254.doi:10.1186/s12879-015-0925-9

7. Garau J, Baquero F, Pérez-Trallero E, Pérez JL, Martín-Sánchez AM, García-Rey C, et al. Factors impacting on length of stay and mortality of community-acquired pneumonia. Clin Microbiol Infect. 2008;14(4):322-9.doi:10.1111/j.1469-0691.2007.01915.x

8. Webb BJ, Sorensen J, Jephson A, Mecham I, Dean NC. Broad-spectrum antibiotic use and poor outcomes in community-onset pneumonia: a cohort study. Eur Respir J. 2019;54(1).doi:10.1183/13993003.00057-2019

9. Bell BG, Schellevis F, Stobberingh E, Goossens H, Pringle M. A systematic review and meta-analysis of the effects of antibiotic consumption on antibiotic resistance. BMC infectious diseases. 2014;14(1):13

10. STATENS SERUM INSTITUT National Food Institute TUoD. DANMAP 2021 Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. DK-2300 Copenhagen; 2021.

11. Sygehusmedicin RfAaD. Baggrundsnotat for hensigtsmæssig anvendelse af antibiotika ved nedre luftvejsinfektioner i almen praksis og på hospital: Rådet for Anvendelse af Dyr Sygehusmedicin; 2016 [accessed on 2021 August 08]. Available from: http://www.regioner.dk/media/3996/bgn-antibiotika-nedre-luftvejsinf-vers-1-0-november-2016-267967.pdf.

12. Rosenvinge FS. Antibiotikavejledning for Region Syddanmark: The region of Southern Denmark 06.10.2021 [accessed on 2022 September 22]. Available from: https://ekstern.infonet.regionsyddanmark.dk/Files/Dokument547684.htm.

13. Mandell LA. Community-acquired pneumonia: An overview. Postgrad Med. 2015;127(6):607-15.doi:10.1080/00325481.2015.1074030

14. Savvateeva EN, Rubina AY, Gryadunov DA. Biomarkers of Community-Acquired Pneumonia: A Key to Disease Diagnosis and Management. Biomed Res Int. 2019;2019:1701276.doi:10.1155/2019/1701276

15. Chandra A, Nicks B, Maniago E, Nouh A, Limkakeng A. A multicenter analysis of the ED diagnosis of pneumonia. The American journal of emergency medicine. 2010;28(8):862-5

16. Garcia-Vazquez E, Marcos MA, Mensa J, de Roux A, Puig J, Font C, et al. Assessment of the usefulness of sputum culture for diagnosis of community-acquired pneumonia using the PORT predictive scoring system. Arch Intern Med. 2004;164(16):1807-11

17. Ewig S, Schlochtermeier M, Goke N, Niederman MS. Applying sputum as a diagnostic tool in pneumonia: limited yield, minimal impact on treatment decisions. Chest. 2002;121(5):1486-92

18. Murphy CN, Fowler R, Balada-Llasat JM, Carroll A, Stone H, Akerele O, et al. Multicenter Evaluation of the BioFire FilmArray Pneumonia/Pneumonia Plus Panel for Detection and Quantification of Agents of Lower Respiratory Tract Infection. J Clin Microbiol. 2020;58(7).doi:10.1128/jcm.00128-20

19. Musher DM, Thorner AR. Community-acquired pneumonia. N Engl J Med. 2014;371(17):1619-28.doi:10.1056/NEJMra1312885

20. Gadsby NJ, Russell CD, McHugh MP, Mark H, Conway Morris A, Laurenson IF, et al. Comprehensive Molecular Testing for Respiratory Pathogens in Community-Acquired Pneumonia. Clin Infect Dis. 2016;62(7):817-23.doi:10.1093/cid/civ1214

21. Gastli N, Loubinoux J, Daragon M, Lavigne JP, Saint-Sardos P, Pailhoriès H, et al. Multicentric evaluation of BioFire FilmArray Pneumonia Panel for rapid bacteriological documentation of pneumonia. Clin Microbiol Infect. 2021;27(9):1308-14.doi:10.1016/j.cmi.2020.11.014

22. Yoo IY, Huh K, Shim HJ, Yun SA, Chung YN, Kang OK, et al. Evaluation of the BioFire FilmArray Pneumonia Panel for rapid detection of respiratory bacterial pathogens and antibiotic resistance genes in sputum and endotracheal aspirate specimens. Int J Infect Dis. 2020;95:326-31.doi:10.1016/j.ijid.2020.03.024

23. Webber DM, Wallace MA, Burnham CA, Anderson NW. Evaluation of the BioFire FilmArray Pneumonia Panel for Detection of Viral and Bacterial Pathogens in Lower Respiratory Tract Specimens in the Setting of a Tertiary Care Academic Medical Center. J Clin Microbiol. 2020;58(7).doi:10.1128/jcm.00343-20

24. Rand KH, Beal SG, Cherabuddi K, Couturier B, Lingenfelter B, Rindlisbacher C, et al. Performance of a Semiquantitative Multiplex Bacterial and Viral PCR Panel Compared With Standard Microbiological Laboratory Results: 396 Patients Studied With the BioFire Pneumonia Panel. Open Forum Infect Dis. 2021;8(1):ofaa560.doi:10.1093/ofid/ofaa560

25. Shengchen D, Gu X, Fan G, Sun R, Wang Y, Yu D, et al. Evaluation of a molecular point-of-care testing for viral and atypical pathogens on intravenous antibiotic duration in hospitalized adults with lower respiratory

tract infection: a randomized clinical trial. Clin Microbiol Infect. 2019;25(11):1415-21.doi:10.1016/j.cmi.2019.06.012

26. Buchan BW, Windham S, Balada-Llasat JM, Leber A, Harrington A, Relich R, et al. Practical Comparison of the BioFire FilmArray Pneumonia Panel to Routine Diagnostic Methods and Potential Impact on Antimicrobial Stewardship in Adult Hospitalized Patients with Lower Respiratory Tract Infections. J Clin Microbiol. 2020;58(7).doi:10.1128/jcm.00135-20

27. Serigstad S, Markussen D, Grewal HMS, Ebbesen M, Kommedal Ø, Heggelund L, et al. Rapid syndromic PCR testing in patients with respiratory tract infections reduces time to results and improves microbial yield. Sci Rep. 2022;12(1):326.doi:10.1038/s41598-021-03741-7

28. Skjøt-Arkil H, Heltborg A, Lorentzen MH, Cartuliares MB, Hertz MA, Graumann O, et al. Improved diagnostics of infectious diseases in emergency departments: a protocol of a multifaceted multicentre diagnostic study. BMJ open. 2021;11(9):e049606

29. Moher D, Hopewell S, Schulz KF, Montori V, Gøtzsche PC, Devereaux PJ, et al. CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. BMJ. 2010;340:c869.doi:10.1136/bmj.c869

30. KL. Udvikling i indlæggelsestid på somatiske hospitaler 2009-2018 2020 [accessed on 2023 May 5]. Available from: https://www.kl.dk/nyheder/momentum/2020/2020-16/de-aeldste-patienter-er-indlagt-i-markant-kortere-tid/.

31. BioFire. 2018. FilmArray Pneumonia panel instruction booklet RFIT-ASY0144/145. BioFire SLC, UT.

32. Harris PA, Taylor R, Minor BL, Elliott V, Fernandez M, O'Neal L, et al. The REDCap consortium: Building an international community of software platform partners. J Biomed Inform. 2019;95:103208.doi:10.1016/j.jbi.2019.103208

33. Hagen TL, Hertz MA, Uhrin GB, Dalager-Pedersen M, Schønheyder HC, Nielsen H. Adherence to local antimicrobial guidelines for initial treatment of community-acquired infections. Dan Med J. 2017;64(6)

34. Plesner LL, Iversen AKS, Langkjær S, Nielsen TL, Østervig R, Warming PE, et al. The formation and design of the TRIAGE study-baseline data on 6005 consecutive patients admitted to hospital from the emergency department. Scand J Trauma Resusc Emerg Med. 2015;23(1):1-9

35. Edin A, Eilers H, Allard A. Evaluation of the Biofire Filmarray Pneumonia panel plus for lower respiratory tract infections. Infect Dis (Lond). 2020;52(7):479-88.doi:10.1080/23744235.2020.1755053

36. Echavarría M, Marcone DN, Querci M, Seoane A, Ypas M, Videla C, et al. Clinical impact of rapid molecular detection of respiratory pathogens in patients with acute respiratory infection. J Clin Virol. 2018;108:90-5.doi:10.1016/j.jcv.2018.09.009

37. Yang S, Li H, Tang Y, Yu F, Ma C, Zhang H, et al. Multiplex Tests for Respiratory Tract Infections: The Direct Utility of the FilmArray Respiratory Panel in Emergency Department. Can Respir J. 2020;2020:6014563.doi:10.1155/2020/6014563

38. von Baum H, Welte T, Marre R, Suttorp N, Ewig S. Community-acquired pneumonia through Enterobacteriaceae and Pseudomonas aeruginosa: Diagnosis, incidence and predictors. Eur Respir J. 2010;35(3):598-605.doi:10.1183/09031936.00091809

39. Institut SS. Influenza season 2020-2021 - report on disease occurrence 2021 [accessed on 2022 August 29]. Available from: https://en.ssi.dk/surveillance-and-preparedness/surveillance-in-denmark/annual-reports-on-disease-incidence/influenza-season-2020-2021---report-on-disease-occurrence.

40. Nagendra S, Bourbeau P, Brecher S, Dunne M, LaRocco M, Doern G. Sampling variability in the microbiological evaluation of expectorated sputa and endotracheal aspirates. J Clin Microbiol. 2001;39(6):2344-7.doi:10.1128/jcm.39.6.2344-2347.2001

41. Cartuliares MB, Rosenvinge FS, Mogensen CB, Skovsted TA, Andersen SL, Pedersen AK, et al. Expiratory Technique versus Tracheal Suction to Obtain Good-Quality Sputum from Patients with Suspected Lower Respiratory Tract Infection: A Randomized Controlled Trial. Diagnostics (Basel). 2022;12(10).doi:10.3390/diagnostics12102504

42. Vest-Hansen B, Riis AH, Sørensen HT, Christiansen CF. Out-of-hours and weekend admissions to Danish medical departments: admission rates and 30-day mortality for 20 common medical conditions. BMJ Open. 2015;5(3):e006731.doi:10.1136/bmjopen-2014-006731

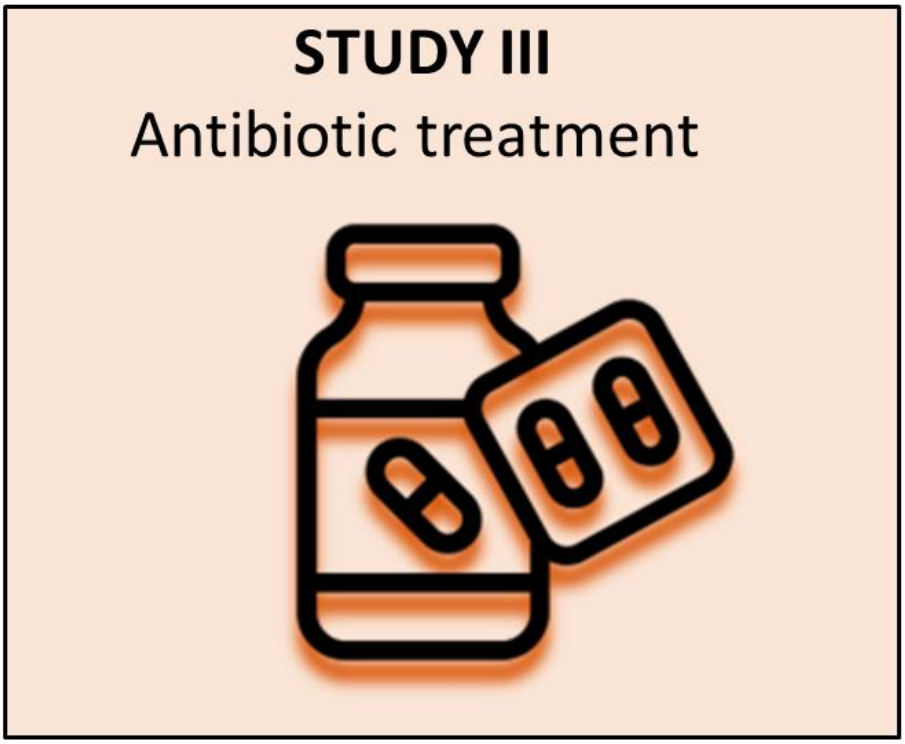
43. Musher DM, Montoya R, Wanahita A. Diagnostic value of microscopic examination of Gram-stained sputum and sputum cultures in patients with bacteremic pneumococcal pneumonia. Clin Infect Dis. 2004;39(2):165-9.doi:10.1086/421497

44. Werner Vach VR, Izabela Kolankowska, Susanne Weber, Gerta Rücker. Design and Evaluation of Diagnostic Studies: Bundensministerium fur Bildung und Forschung; 2017.

45. Hot A, Bossuyt PM, Gerke O, Wahl S, Vach W, Zapf A. Randomized test-treatment studies with an outlook on adaptive designs. BMC Med Res Methodol. 2021;21(1):110.doi:10.1186/s12874-021-01293-y

46. Schouten JA, Hulscher ME, Natsch S, Kullberg BJ, van der Meer JW, Grol RP. Barriers to optimal antibiotic use for community-acquired pneumonia at hospitals: a qualitative study. Qual Saf Health Care. 2007;16(2):143-9.doi:10.1136/qshc.2005.017327

11.3.1 Paper III – Supplementary material



The effect of point-of-care multiplex polymerase chain reaction of respiratory specimens on antibiotic treatment of patients acutely admitted with suspected community-acquired pneumonia in Denmark: A multicentre randomised controlled trial

Authors: Mariana Bichuette Cartuliares [1, 2*],Flemming Schønning Rosenvinge [3,4], Christian Backer Mogensen [1, 2], Thor Aage Skovsted [5], Steen Lomborg Andersen [6], Claus Østergaard [7], Andreas Kristian Pedersen [8], Helene Skjøt-arkil [1, 2]

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Table S1: Empirical treatment guidelines of CAP of the region of Southern Denmark

Severity of CAP	First choice	Penicillin allergy	Therapy duration (iv* and oral)
CURB-65: 0-2	Benzylpenicillin 1.2g (2 mill.IE) x4 iv. or Phenoxymethylpenicillin 0.6g (1 mill.IE) x 4 oral	Cefuroxime 1.5g x 3 iv. or Roxithromycin 300mg x1 oral	5 days
CURB-65 ≥ 3	Benzylpenicillin 1.2g (2 mill.IE) x 4 iv. + Azithromycin† 500mg x 1 iv.	Cefuroxime 1.5g x 3 iv. + Azithromycin 500mg x 1 iv.	7 days
CURB-65 ≥ 3+□	Piperacillin-tazobactam 4/0.5gx3 iv. + Azithromycin 500mg x1 iv.	Cefuroxime 1.5g x 3 iv. + Azithromycin 500mg x 1 iv.	7 days

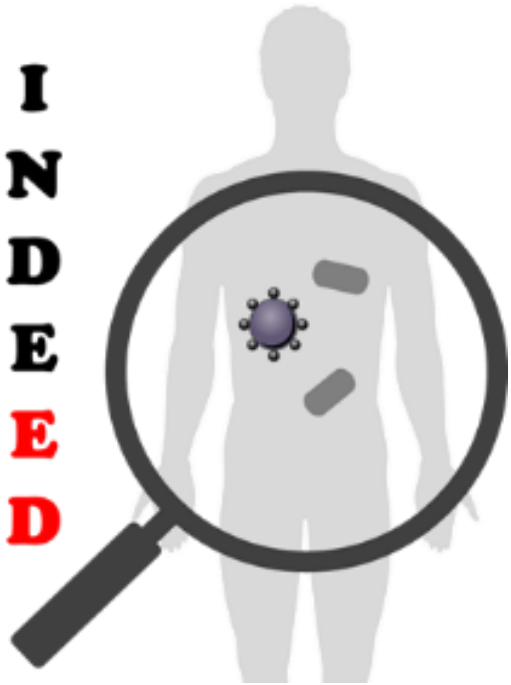
*Intravenous route. † Azithromycin: The treatment is extended only if PCR is positive for *Legionella pneumophila*, *Mycoplasma pneumoniae*, or *Chlamydophila pneumoniae*. Azithromycin (500 mg iv.) has approx. 2-4 days therapeutic coverage. □CURB-65 ≥ 3+: Confusion, urea, respiratory frequency, blood pressure + radiological involvement of multiple lung lobes, or hypoxia with O_2 saturation < 92%, or sepsis.

Table S2: Standard care procedures in our emergency departments

TIMEPOINT	30 min.	Within 1-hour	Within 4-hours	48-hours	Day 5
Clinical assessment	x				
Tracheal secretions /sputum collection		x			
Microbiological results				x	
Blood samples collection		x			
Biochemistry results			x		
Chest X-ray			x		
Empirical treatment			x		
Adjustment of therapy				x	x

Text S3: Action card

Guidance of results from POC-PCR



This guidance is developed to the INDEED-study (Infectious diseases in Emergency Department).

Emergency department physicians from Hospital Sønderjylland in Aabenraa, Hospital Lillebælt in Kolding, and Odense University Hospital in Odense, will receive this action card along with the results from sputum sample analyses.

In case of doubt in the interpretation of the results, the physician is encouraged to contact the local clinical microbiologist.

Agens	Association with CAP[#]	Remarks	Antibiotics	
			First choice	Penicillin allergy
Streptococcus pneumoniae *	Frequent and likely pathogen	Part of the normal microbiota in upper respiratory tract. May be contamination with pharyngeal microbiota.	Benzylpenicillin 1.2g (2 mill.IE) x4 i.v. *or* Phenoxymethylpenicillin 0.6g (1 mill.IE) x4 oral	Cefuroxime 1.5g x 3 i.v. *or* Roxithromycin 300mg x1 oral
*Haemophilus influenza**	Frequent and likely pathogen		Ampicillin 2g x4 i.v. *or* Benzylpenicillin 1.2g (2 mill. IE) x4 i.v. *or* Piv-ampicillin 1g x3 oral *or* Amoxicillin 1g x3 oral	Cefuroxime 1.5g x 3 i.v. *or* Doxycycline 100mg x2 first 24 hours oral followed by 100mg x1 oral
*Streptococcus pyogenes**	Probable, but rare pathogen	Part of the normal microbiota in upper respiratory tract. These pathogens relatively often represent contamination with pharyngeal microbiota. Infection caused by *Streptococcus pyogenes* or *Staphylococcus aureus* will	Benzylpenicillin 1.2g (2 mill. IE) x4 i.v.	Cefuroxime 1.5g x3 i.v.
*Streptococcus agalactiae**	Rare pathogen in adults		Benzylpenicillin 1.2g (2 mill. IE) x4 i.v.	Cefuroxime 1.5g x3 i.v.
*Staphylococcus aureus**	Probable, but rare pathogen		Cloxacillin 1g x4 i.v.	Cefuroxime 1.5g x3 i.v.
*Moraxella catarrhalis**	Probable pathogen		Piperacillin-tazobactam 4/0.5g x3 i.v. *or* amoxicillin-clavulanic acid 500/125mg x3 oral	Cefuroxime 1.5g x3 i.v. *or* Roxithromycin 300mg x1 oral *or* Azithromycin 500mg x1 oral

		usually results in severe pneumonia.		
Legionella pneumophila Mycoplasma pneumonia	Likely causative pathogen	Is not a part of the normal respiratory microbiota.	Azithromycin 500mg x1 i.v./oral	
Chlamydia pneumoniae	Probable causative pathogen	Is not a part of the normal respiratory microbiota Will usually cause mild infections. In case of severe infection, other pathogens/super-infection should be considered.	Azithromycin 500mg x1 i.v./oral	

Agens	Association with CAP[#]	Remarks	Antibiotics
*Pseudomonas aeruginosa** *Acinetobacter calcoaceticus-baumannii complex** *Enterobacter cloacae** *Escherichia coli** *Klebsiella (Enterobacter) aerogenes** *Klebsiella oxytoca** *Klebsiella pneumoniae group** *Proteus spp.** *Serratia marcescens**	Very rare causative pathogens	These findings usually represents colonization.	These findings should typically not lead to adjustment of empirical antimicrobial treatment.
Influenza A Influenza B	Frequent pathogens	Is not a part of the normal respiratory microbiota Bacterial superinfection can occur.	Consider whether the patient's pneumonia symptoms can be explained by viral infection, and whether antibiotic treatment is necessary / indicated.
Parainfluenza virus Respiratory Syncytial Adenovirus Coronavirus *(does not include SARS-CoV-2)* Human Rhinovirus/Enterovirus Human Metapneumovirus	Probable pathogens	Usually causes mild infections. In case of severe infection, other pathogens / superinfection should be considered. May be an accidental finding due to previous /recent / asymptomatic infection.	
Not detected	A negative result does not rule out pneumonia, but means that CAP caused by the most common		

(POC-PCR(FilmArray) is negative)	pathogens is less likely. Consider whether the pneumonia diagnosis is correct and consider investigation for rare causes of pneumonia (e.g. tubeculosis or *Chlamydia psittaci*).

#CAP: Community-Acquired Pneumonia

*: Concentration (copies/mL) is reported in the POC-PCR (FilmArray) result

Most bacterial causative pathogens of CAP are also part of the normal respiratory microbiota or may colonize the upper respiratory tract, and the clinical relevance of these findings must always be assessed carefully.

For the bacterial agents marked with "*", a concentration (copies/mL) is reported in the POC-PCR (FilmArray) result. There is a reasonable correlation between copies/mL and the culture-based measure "CFU/mL", however, "copies/mL" is typically a factor of 10-100 higher than the corresponding "CFU/mL".

The limits of significance are not well established and depend probably on the agent, the quality of the sample and the clinical context - and must therefore be used with caution. The Infectious Diseases Society of America and the American Society of Microbiology[1] propose the following culture-based limits for hospital-acquired pneumonia:

Culture-based measure	POC-PCR (FilmArray) concentration	Interpretation (caution)
$< 10^4$ CFU/mL	≈ $< 10^5$ copies/mL	Indicates mixture with normal flora
$10^4 – 10^5$ CFU/mL	≈ 10^5-10^6 copies/mL	Gray zone
$> 10^5$ CFU/mL	≈ $>10^6$ copies/mL	Indicates real findings

Developed by microbiologist Flemming Rosenvinge, Department of Clinical Microbiology, Odense, University Hospital in Odense, and microbiologist Claus Østergaard, Department of Clinical

Microbiology, Hospital Lillebælt in Kolding, Denmark

Version 1.1 – February 7th 2021

[1] *Miller, J. M., Binnicker, M. J., Campbell, S., et al. A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases: 2018 Update by the Infectious Diseases Society of America and the American Society for Microbiology. Clinical Infectious Diseases, 67(6), e1–e94. https://doi.org/10.1093/cid/ciy381*

Table S3: Targets of the Biofire® FilmArray® Pneumonia Panel plus (Biomérieux, Marcy l'Etoile, France)

Bacteria	Atypical bacteria	Antimicrobial resistance genes
Acinetobacter calcoaceticus baumannii complex	*Chlamydophilia pneumoniae*	*mecA/C and MREJ*
Enterobacter cloacae complex	*Legionella pneumophila*	*KPC,*
Escherichia coli	*Mycoplasma pneumonia*	*CTX-M*
Haemophilus influenzae		*NDM*
Klebsiella aerogenes		*Oxa48-like*
Klebsiella oxytoca	Viruses	*VIM*
Klebsiella pneumoniae group	*Influenza A*	*IMP*
Moraxella catarrhalis	*Influenza B*	
Proteus spp.	*Adenovirus**	
Pseudomonas aeruginosa	*Parainfluenza virus*	
Serratia marcescens	*Coronavirus (CoV)***	
Staphylococcus aureus	*Human metapneumovirus*	
Streptococcus agalactiae,	*Human rhinovirus/enterovirus*	
Streptococcus pneumoniae	*MERS-CoV*	
Streptococcus pyogenes	*Respiratory syncytial virus*	

* Adenovirus is not included in our analysis due to the expiration date specific for Adenovirus announced
by Biofire Nordic 21. July 2021. Biofire® FilmArray® Pneumonia plus (PN plus) Panel (RFIT-ASY-0142 and RFIT-ASY-0143).
** Coronavirus (CoV): serological variants (229E, OC43, HKU1, NL63)

Table S4: Classification of "Narrow antibiotic" treatment

Classification	Antibiotic	CAVE
Narrow-spectrum antibiotics against CAP and	Benzylpenicillin Phenoxymethylpenicillin Ampicillin Pivampicillin Amoxicillin	No
	Macrolides Cefuroxime	Yes
No antibiotics	-	-
	<u>*Monotherapy:*</u> Amoxicillin/Clavulanic acid' Piperacillin/Tazobactam Doxycycline Tetracycline	No

Broad-spectrum antibiotics and antibiotics not directed against CAP	Moxifloxacin Sulfamethoxazole and trimethoprim Macrolides	
	Cefuroxime	Is considered narrow in case of CAVE
	Combination therapy: Benzylpenicillin *or* Phenoxymethylpenicillin *or* Ampicillin *or* Pivampicillin *or* Amoxicillin *or* Amoxicillin/Clavulanic acid'*or* Tazobactam/Piperacillin *or* Cefuroxim *Combined with:* Doxycylin *or* Tetracyclin *or* Ciprofloxacin *or* Moxifloxacin *or* Macrolides *Antibiotics not directed against CAP:* Dicloxacillin Cloxacillin Flucloxacillin Pivmecillinam Mecillinam Tigecyclin Cefalexin Cefazolin Cefotaxim Ceftazidim Ceftriaxon Cefepime	No

	Ceftolozan/Tazobactam Ceftazidim/Avibactam Meropenem Ertapenem Imipenem- cilastatin Trimethoprim Sulfamethizol Tobramycin Gentamicin Clindamycin Ciprofloxacin	

Macrolides* = Erythromycin or roxithromycin or clarithromycin or azithromycin

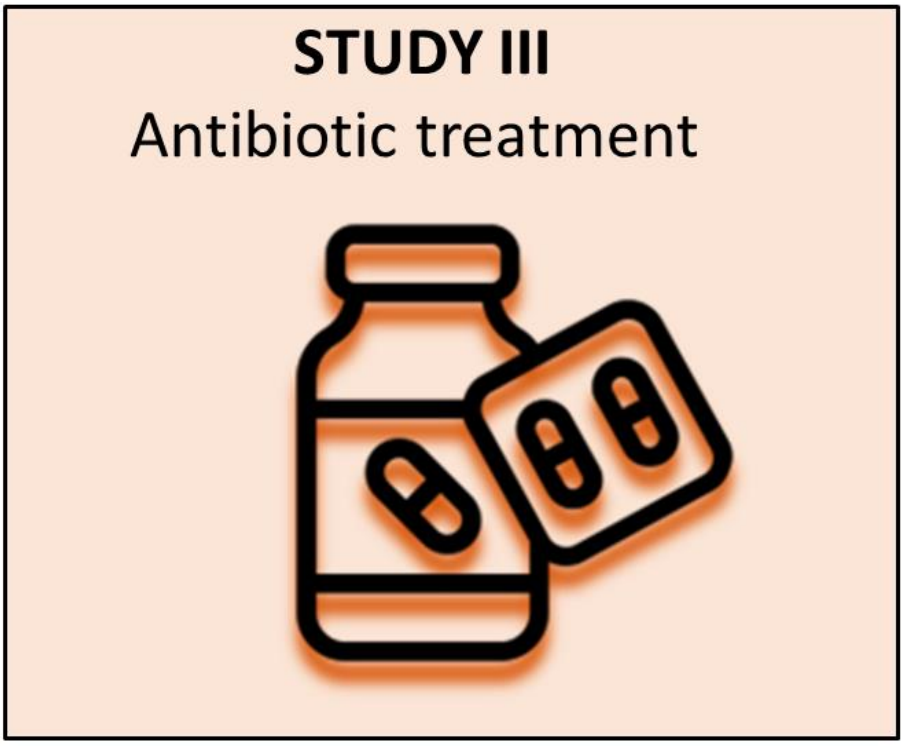
Table S5: Classification of ”targeted and adequate” treatment

Antimicrobial	Microbiological agents						
	S.Pneumo-niae	H.influen-zae	M.cata-rrhalis	P.aerugi-nosa	S. aureus	Hem. Strepto-coccus	L. pneumo-phila
Benzylpenicillin	Targeted	Targeted	not relevant	not relevant	not relevant	Targeted	not relevant
Phenoxymethyl-penicillin	Targeted	not relevant	not relevant	not relevant	not relevant	Targeted	not relevant
Ampicillin	Adequate	Targeted	not relevant	not relevant	not relevant	Adequate	not relevant
Pivampicillin	Adequate	Targeted	not relevant	not relevant	not relevant	Adequate	not relevant
Amoxicillin	Adequate	Targeted	not relevant	not relevant	not relevant	Adequate	not relevant
Pivmecillinam	not relevant	not relevant	not relevant	not relevant	not relevant	not relevant	not relevant
Mecillinam	not relevant	not relevant	not relevant	not relevant	not relevant	not relevant	not relevant
Dicloxacillin	not relevant	not relevant	not relevant	not relevant	Targeted	not relevant	not relevant
Cloxacillin	not relevant	not relevant	not relevant	not relevant	Targeted	not relevant	not relevant
Flucloxacillin	not relevant	not relevant	not relevant	not relevant	Targeted	not relevant	not relevant
Amoxicillin/ Clavulanic acid’	Adequate	Targeted	Targeted	not relevant	Adequate	Adequate	not relevant
Tazobactam/ Piperacillin	Adequate	Targeted	Targeted	Targeted	Adequate	Adequate	not relevant
Cefuroxime	CAVE/ Targeted	CAVE/ Targeted	Targeted	not relevant	CAVE/ Targeted	CAVE/ Targeted	not relevant
Cefotaxim	Adequate	Adequate	Adequate	not relevant	Adequate	Adequate	not relevant
Ceftriaxon	Adequate	Adequate	Adequate	not relevant	Adequate	Adequate	not relevant

Ceftazidim	not relevant	Adequate	Adequate	Targeted	not relevant	Adequate	not relevant
Cefepime	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate	not relevant
Meropenem	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate	not relevant
Ertapenem	Adequate	Adequate	Adequate	not relevant	Adequate	Adequate	not relevant
Imipenem and cilastatin	Adequate	Adequate	Adequate	Adequate	Adequate	Adequate	not relevant
Macrolides*	CAVE/ Targeted	not relevant	Targeted	not relevant	not relevant	CAVE/ Targeted	Targeted
Clindamycin	CAVE/ Targeted	not relevant	not relevant	not relevant	CAVE/ Targeted	CAVE/ Targeted	not relevant
Doxycylin	Adequate	CAVE/ Targeted	Adequate	not relevant	Adequate	Adequate	Targeted
Tetracyclin	Adequate	CAVE/ Targeted	Adequate	not relevant	Adequate	Adequate	Targeted
Tigecyclin	Adequate	Adequate	Adequate	not relevant	Adequate	Adequate	not relevant
Tobramycin	not relevant	not relevant	not relevant	Targeted	not relevant	not relevant	not relevant
Gentamicin	not relevant	not relevant	not relevant	Targeted	not relevant	not relevant	not relevant
Ciprofloxacin	not relevant	CAVE/ Targeted	Adequate	Targeted	not relevant	not relevant	Targeted
Moxifloxacin	Adequate	Adequate	Adequate	not relevant	Adequate	Adequate	Targeted
Trimethoprim	not relevant	not relevant	not relevant	not relevant	not relevant	not relevant	not relevant
Sulfamethizol	not relevant	not relevant	not relevant	not relevant	not relevant	not relevant	not relevant
Sulfamethoxazole and trimethoprim	Adequate	Adequate	Adequate	not relevant	Adequate	Adequate	not relevant

Green (targeted treatment): Antibiotics directed against a bacterial pathogen detected by culture without being unnecessary board-spectrum.
Blue (CAVE/Targeted): Considered targeted treatment if the patient was registered as allergic to penicillins.
Yellow (Adequate): Antibiotics that are active against the bacterial pathogen detected by culture.
Orange (Not relevant): Antibiotics that are not recommended and/or regarded inactive against the bacterial pathogen detected by culture.
*Macrolides: Erythromycin or roxithromycin or clarithromycin or azithromycin.

11.3.2 Paper III – CONSORT Checklist



CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	1-2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	2-3
	2b	Specific objectives or hypotheses	4
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	4
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	n/a
Participants	4a	Eligibility criteria for participants	5
	4b	Settings and locations where the data were collected	5
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	6-7

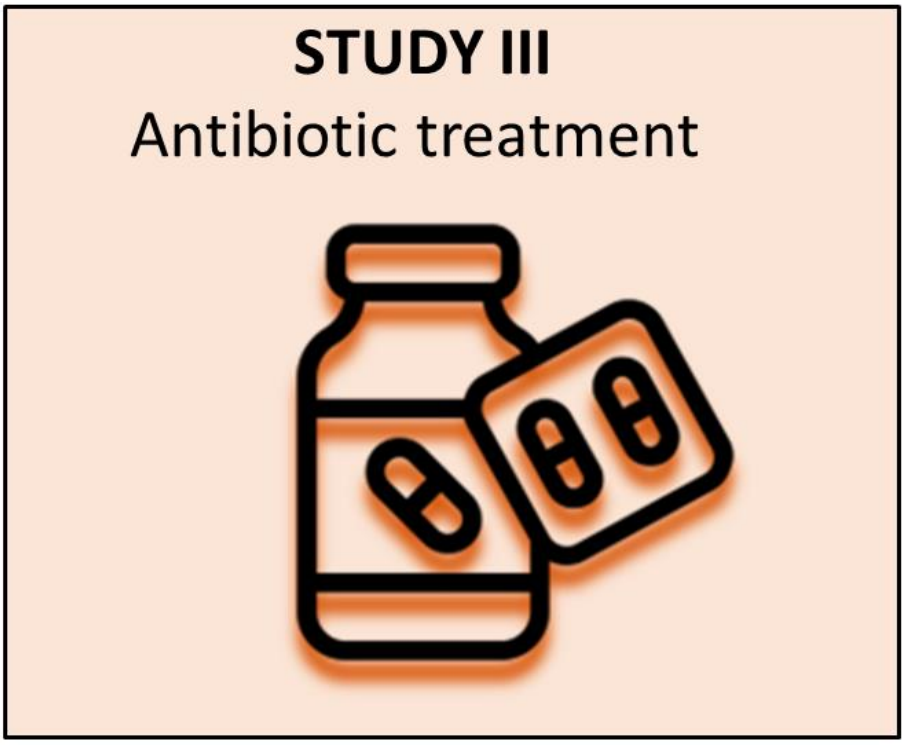
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	8
	6b	Any changes to trial outcomes after the trial commenced, with reasons	9
Sample size	7a	How sample size was determined	9
	7b	When applicable, explanation of any interim analyses and stopping guidelines	n/a
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	5-6
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	5-6
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	5-6
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	6
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	6
	11b	If relevant, description of the similarity of interventions	n/a

Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	9
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	9
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	10
	13b	For each group, losses and exclusions after randomisation, together with reasons	10
Recruitment	14a	Dates defining the periods of recruitment and follow-up	4
	14b	Why the trial ended or was stopped	n/a
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	11+12
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	10
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	12+13 tables 2+3 and figure 2
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	Table S5 appendix

Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	Table 2+3
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	Table 3
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	18
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	18
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	18
Other information			
Registration	23	Registration number and name of trial registry	4
Protocol	24	Where the full trial protocol can be accessed, if available	4
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	19

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

11.3.3 Paper III - Data availability and data sharing plan



Data availability and data sharing plan

Anonymized personal data is not subject to data protection legislation by the General Data Protection Regulation (GDPR) in the EU and is therefore allowed to be publicly shared. However, the personal data underlying the results in the article is not possible fully anonymize and is therefore covered by § 10 of the Danish Data Protection Act.

When personal data covered by Section 10 of the Data Protection Act (also applies to pseudonymized information) wishes to be passed on with a view to publication in a recognized scientific journal, it requires permission from the Danish Data Protection Authority, cf. Section 10, subsection of the Data Protection Act. 3, No. 3. However, the Danish Data Protection Authority can only approve this sharing if there is an authority in the informed consent from the ethical approval cf. Section 2, subsection 10 of the Danish Committees Act. In the ethical approval, S-20200188 underlying this project is it stated that personal data is anonymized upon publication. It is, therefore, not possible to share pseudonymized information unrestricted.

Upon request, can the project Sponsor Christian Backer Mogensen apply The Regional Committees on Health Research Ethics for Southern Denmark for a supplement to the Ethical protocol describing the relevance of the transferal of personal data to an additional partner. The request can be sent to Fortegnelsen-SHS@rsyd.dk Special consultent Signe Bek Sørensen Kresten Philipsensvej 15, 6200 Aabenraa, Denmark.

Data sharing statement	
Will individual deidentified participant data (including data dictionaries) will be shared	Upon request
What data in particular will be shared	Pseudonymized participant data that underlie the results reported in this article, after deidentification (text, tables, figures, and supplementary material).
What other documents will be available	Study protocol, Statistical Analysis plan, Informed consent form.
When will data be available	Beginning 3 months and ending 5 years following article publication.
With whom	Investigators and researchers whose proposed use of the data has been approved by an independent review committee identified for this purpose.
For what types of analyses	To achieve the aims in the approved proposal and for individual participant data meta-analysis.
By what mechanism will the data be available	Proposals should request the data to Fortegnelsen-SHS@rsyd.dk where the data will be available for 5 years.

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